AO 440 (Rev. 8/01) Summons in a Civil Action

UNITED STATES DISTRICT COURT

Eastern District of Virginia

PHOTOCURE ASA

V.

JON W. DUDAS,
Under Secretary of Commerce for
Intellectual Property and
Director of the United States
Patent and Trademark Office
and JOHN J. DOLL,
Commissioner for Patents,
United States Patent and
Trademark Office

SUMMONS IN A CIVIL CASE

CASE NUMBER: 1:08CV718

RECEIVED
2008 JUL 15 AM 12:09
U.S. ATTORNEY'S OFFICE
ALEXANDRIA, VA

TO: (Name and address of Defendant)
Jon W. Dudas
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office
c/o Office of the General Counsel
United States Patent and Trademark Office
Madison Building East, Room 10B20
600 Delany Street
Alexandria, VA 22314

YOU ARE HEREBY SUMMONED and required to serve on PLAINTIFF'S ATTORNEY (name and address)
Erik Kane
Kenyon & Kenyon LLP
1500 K Street NW
Suite 700
Washington DC 20005

an answer to the complaint which is served on you with this summons, within 60 days after service of this summons on you, exclusive of the day of service. If you fail to do so, judgment by default will be taken against you for the relief demanded in the complaint. Any answer that you serve on the parties to this action must be filed with the Clerk of this Court within a reasonable period of time after service.

Fernando Galindo, Clerk
CLERK

July 14, 2008
DATE

Tina L. Fitzgerald
(By) DEPUTY CLERK

AO 440 (Rev. 8/01) Summons in a Civil Action

RETURN OF SERVICE

Service of the Summons and complaint was made by me[1]	DATE
NAME OF SERVER *(PRINT)*	TITLE

Check one box below to indicate appropriate method of service

☐ Served personally upon the defendant. Place where served: _____

☐ Left copies thereof at the defendant's dwelling house or usual place of abode with a person of suitable age and discretion then residing therein.

Name of person with whom the summons and complaint were left: _____

☐ Returned unexecuted: _____

☐ Other (specify): _____

STATEMENT OF SERVICE FEES

TRAVEL	SERVICES	TOTAL

DECLARATION OF SERVER

I declare under penalty of perjury under the laws of the United States of America that the foregoing information contained in the Return of Service and Statement of Service Fees is true and correct.

Executed on _____ _____
Date *Signature of Server*

_____
Address of Server

(1) As to who may serve a summons see Rule 4 of the Federal Rules of Civil Procedure.

JS 44 (Rev. 12/07)

CIVIL COVER SHEET

RECEIVED

The JS 44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

I. (a) PLAINTIFFS Photocure ASA

(b) County of Residence of First Listed Plaintiff n/a - Foreign Entity
(EXCEPT IN U.S. PLAINTIFF CASES)

(c) Attorney's (Firm Name, Address, and Telephone Number)
Kenyon & Kenyon LLP, 1500 K Street, NW
Washington DC 20005, (202)220-4200, Erik C. Kane

DEFENDANTS United States Patent & Trademark Office
Dudas, Jon W., Director and Under Secretary of Commerce for Intellectual Property
Doll, John J., Commissioner for Patents

2008 JUL 11 P 1:52

CLERK US DISTRICT COURT
ALEXANDRIA, VIRGINIA

County of Residence of First Listed Defendant
(IN U.S. PLAINTIFF CASES ONLY)
NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE LAND INVOLVED.

Attorneys (If Known)

II. BASIS OF JURISDICTION (Place an "X" in One Box Only)

- ☐ 1 U.S. Government Plaintiff
- ☐ 3 Federal Question (U.S. Government Not a Party)
- ☒ 2 U.S. Government Defendant
- ☐ 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (Place an "X" in One Box for Plaintiff and One Box for Defendant)
(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	☐ 1	☐ 1	Incorporated *or* Principal Place of Business In This State	☐ 4	☐ 4
Citizen of Another State	☐ 2	☐ 2	Incorporated *and* Principal Place of Business In Another State	☐ 5	☐ 5
Citizen or Subject of a Foreign Country	☐ 3	☐ 3	Foreign Nation	☐ 6	☐ 6

IV. NATURE OF SUIT (Place an "X" in One Box Only)

CONTRACT
- ☐ 110 Insurance
- ☐ 120 Marine
- ☐ 130 Miller Act
- ☐ 140 Negotiable Instrument
- ☐ 150 Recovery of Overpayment & Enforcement of Judgment
- ☐ 151 Medicare Act
- ☐ 152 Recovery of Defaulted Student Loans (Excl. Veterans)
- ☐ 153 Recovery of Overpayment of Veteran's Benefits
- ☐ 160 Stockholders' Suits
- ☐ 190 Other Contract
- ☐ 195 Contract Product Liability
- ☐ 196 Franchise

REAL PROPERTY
- ☐ 210 Land Condemnation
- ☐ 220 Foreclosure
- ☐ 230 Rent Lease & Ejectment
- ☐ 240 Torts to Land
- ☐ 245 Tort Product Liability
- ☐ 290 All Other Real Property

TORTS

PERSONAL INJURY
- ☐ 310 Airplane
- ☐ 315 Airplane Product Liability
- ☐ 320 Assault, Libel & Slander
- ☐ 330 Federal Employers' Liability
- ☐ 340 Marine
- ☐ 345 Marine Product Liability
- ☐ 350 Motor Vehicle
- ☐ 355 Motor Vehicle Product Liability
- ☐ 360 Other Personal Injury

PERSONAL INJURY
- ☐ 362 Personal Injury - Med. Malpractice
- ☐ 365 Personal Injury - Product Liability
- ☐ 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- ☐ 370 Other Fraud
- ☐ 371 Truth in Lending
- ☐ 380 Other Personal Property Damage
- ☐ 385 Property Damage Product Liability

CIVIL RIGHTS
- ☐ 441 Voting
- ☐ 442 Employment
- ☐ 443 Housing/ Accommodations
- ☐ 444 Welfare
- ☐ 445 Amer. w/Disabilities - Employment
- ☐ 446 Amer. w/Disabilities - Other
- ☐ 440 Other Civil Rights

PRISONER PETITIONS
- ☐ 510 Motions to Vacate Sentence

Habeas Corpus:
- ☐ 530 General
- ☐ 535 Death Penalty
- ☐ 540 Mandamus & Other
- ☐ 550 Civil Rights
- ☐ 555 Prison Condition

FORFEITURE/PENALTY
- ☐ 610 Agriculture
- ☐ 620 Other Food & Drug
- ☐ 625 Drug Related Seizure of Property 21 USC 881
- ☐ 630 Liquor Laws
- ☐ 640 R.R. & Truck
- ☐ 650 Airline Regs.
- ☐ 660 Occupational Safety/Health
- ☐ 690 Other

LABOR
- ☐ 710 Fair Labor Standards Act
- ☐ 720 Labor/Mgmt. Relations
- ☐ 730 Labor/Mgmt.Reporting & Disclosure Act
- ☐ 740 Railway Labor Act
- ☐ 790 Other Labor Litigation
- ☐ 791 Empl. Ret. Inc. Security Act

IMMIGRATION
- ☐ 462 Naturalization Application
- ☐ 463 Habeas Corpus - Alien Detainee
- ☐ 465 Other Immigration Actions

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- ☐ 422 Appeal 28 USC 158
- ☐ 423 Withdrawal 28 USC 157

PROPERTY RIGHTS
- ☐ 820 Copyrights
- ☒ 830 Patent
- ☐ 840 Trademark

SOCIAL SECURITY
- ☐ 861 HIA (1395ff)
- ☐ 862 Black Lung (923)
- ☐ 863 DIWC/DIWW (405(g))
- ☐ 864 SSID Title XVI
- ☐ 865 RSI (405(g))

FEDERAL TAX SUITS
- ☐ 870 Taxes (U.S. Plaintiff or Defendant)
- ☐ 871 IRS—Third Party 26 USC 7609

OTHER STATUTES
- ☐ 400 State Reapportionment
- ☐ 410 Antitrust
- ☐ 430 Banks and Banking
- ☐ 450 Commerce
- ☐ 460 Deportation
- ☐ 470 Racketeer Influenced and Corrupt Organizations
- ☐ 480 Consumer Credit
- ☐ 490 Cable/Sat TV
- ☐ 810 Selective Service
- ☐ 850 Securities/Commodities/ Exchange
- ☐ 875 Customer Challenge 12 USC 3410
- ☐ 890 Other Statutory Actions
- ☐ 891 Agricultural Acts
- ☐ 892 Economic Stabilization Act
- ☐ 893 Environmental Matters
- ☐ 894 Energy Allocation Act
- ☐ 895 Freedom of Information Act
- ☐ 900Appeal of Fee Determination Under Equal Access to Justice
- ☐ 950 Constitutionality of State Statutes

V. ORIGIN (Place an "X" in One Box Only)

☒ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from another district (specify) ☐ 6 Multidistrict Litigation ☐ 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION

Cite the U.S. Civil Statute under which you are filing (Do not cite jurisdictional statutes unless diversity): 5 U.S.C. §702 and 35 U.S.C. §156

Brief description of cause: Review of Defendants' Denial of Plaintiff's Application for Extension of Patent Term

VII. REQUESTED IN COMPLAINT:

☐ CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23

DEMAND $ Judgment Reversing Denial

CHECK YES only if demanded in complaint:
JURY DEMAND: ☐ Yes ☒ No

VIII. RELATED CASE(S) IF ANY

(See instructions): JUDGE _____ DOCKET NUMBER _____

DATE 07/11/2008

SIGNATURE OF ATTORNEY OF RECORD Erik C. Kane

(VSB # 68294)

FOR OFFICE USE ONLY

RECEIPT # _____ AMOUNT _____ APPLYING IFP _____ JUDGE _____ MAG. JUDGE _____

IN THE UNITED STATES DISTRICT COURT FOR THE EASTERN DISTRICT OF VIRGINIA
Alexandria Division

PHOTOCURE ASA,
Plaintiff,

v.

JON W. DUDAS,
Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office,
and JOHN J. DOLL, Commissioner for Patents,

Defendants.

Civil Action No. 1:08CV718-LO/JFA

COMPLAINT FOR DECLARATORY JUDGMENT AND INJUNCTIVE RELIEF

Plaintiff, for its complaint herein, alleges:

NATURE OF THE ACTION

1. This is an action for a declaratory judgment that defendants' decision denying plaintiff's application for extension of a patent term under 35 U.S.C. § 156 is contrary to law, and for injunctive and other relief.

2. Plaintiff seeks review of defendants' denial of plaintiff's application for extension of a patent term under 35 U.S.C. § 156. The decision ("Final Decision") of the Commissioner for Patents, the United States Patent and Trademark Office ("PTO"), dated May 13, 2008, denying plaintiff's application with respect to U.S. Patent No. 6,034,267 (the "'267 patent"), is attached hereto as Exhibit A. A copy of the '267 patent is attached as Exhibit B. A copy of the

defendant Commissioner's initial decision ("Notice of Final Determination"), dated April 11, 2007, is attached as Exhibit C.

3. This Action arises under 35 U.S.C. § 156 (added by the Drug Price Competition and Patent Term Restoration Act), and the Administrative Procedure Act, 5 U.S.C. §§ 701-706.

JURISDICTION AND VENUE

4. This Court has jurisdiction to hear this action and is authorized to issue the relief sought pursuant to 28 U.S.C. §§ 1331, 1338(a), 1361, 2201-2202, and 5 U.S.C. §§ 701-706. There exists between the parties an actual controversy, justiciable in character, in respect of which plaintiff requires a declaration of its rights by the Court.

5. Venue is proper in this district by virtue of 28 U.S.C. § 1391(e).

THE PARTIES

6. Plaintiff Photocure ASA ("Photocure") is a Norwegian public limited company having a place of business at Hoffsveien 48, NO-0377 Oslo, Norway. Plaintiff is the current owner of the '267 patent.

7. Defendant Jon W. Dudas is named in his official capacity as Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office, Patent and Trademark Office, United States Department of Commerce. The Director is the head of the PTO and is responsible for superintending or performing all duties required by law with respect to the granting and issuing of patents, and is designated by statute as the official with responsibility for decisions to grant extension of patent terms under 35 U.S.C. § 156, which

was added by the Drug Price Competition and Patent Term Restoration Act of 1984. *See* 35 U.S.C. §§ 3(a), 156(d) and (e).

8. Defendant John J. Doll is named in his official capacity as Commissioner for Patents, Patent and Trademark Office, United States Department of Commerce. The Commissioner for Patents is the chief operating officer for the operation of the PTO relating to patents and is responsible for the management and direction of all aspects of the activities of the PTO that affect the administration of patent operations. *See* 35 U.S.C. § 3(b)(2)(A). The Final Decision, dated May 13, 2008, denying plaintiff's application for patent term extension for Metvixia™ was issued in the name of the Commissioner for Patents.

THE '267 PATENT AND THE METVIXIA™ DRUG PRODUCT

9. The '267 patent was issued by the United States Patent and Trademark Office on March 7, 2000, naming as inventors Karl E. Gierskcky, Johan Moan, Qian Peng, Harald Steen, Trond Warloe, and Alf Bjorseth. The '267 patent concerns treatment of actinic keratoses, among other disorders, by the technique known as photodynamic therapy ("PDT," also known as "photochemotherapy"). PDT is a medical treatment for eliminating unwanted cells, such as those of a tumor or other growth. PDT entails causing a light-sensitive molecule (a "photosensitizer") to accumulate in the unwanted cells, and then shining light of an appropriate wavelength on the cells, thereby activating the photosensitizer, which sets off a chain of events that culminates in the death of the cells. It is desirable that the photosensitizer accumulate primarily in the unwanted cells and to a lesser extent or not at all in neighboring, normal cells. Actinic keratosis is a premalignant warty lesion that occurs on the sun-exposed skin, *e.g.*, face,

scalp, or hands, of aged light-skinned people. It may develop into squamous cell carcinoma of low-grade malignancy or into basal cell carcinoma.

10. The inventors discovered, among other things, a method of PDT treatment that entails administering methylaminolevulinate hydrochloride to a tissue to be treated, thereby inducing the accumulation of the photosensitizer protoporphyrin IX in the cells to be eliminated. This method affords substantial advantages over the use in PDT of aminolevulinic acid hydrochloride. *See* '267 patent at col. 1, ll. 9-20, col. 3, ll. 19-23, col. 5, ll. 25-32, col. 4, l. 57-col. 5, l. 9. Both methylaminolevulinate and aminolevulinic acid are converted by cells into protoporphyrin IX. The advantages that methylaminolevulinate affords over aminolevulinic acid include superior selectivity of uptake by target lesions, superior penetration of target lesions, reduced (unwanted) systemic distribution of the active ingredient, and reduced pain resulting from PDT. *See id.* at col. 4, l. 57-col. 5, l. 9. In the '267 patent, the inventors were granted claims both to pharmaceutical compositions comprising methylaminolevulinate hydrochloride and to methods of photochemotherapeutic treatment comprising administering such compositions.

11. The commercial embodiment of the '267 patent is Metvixia™, which contains methylaminolevulinate hydrochloride, along with inactive ingredients. While aminolevulinic acid hydrochloride had previously been marketed in the United States in other drug products, Metvixia™ was the first commercial drug product to contain methylaminolevulinate hydrochloride (an ester of aminolevulinic acid hydrochloride). Metvixia™ was a new drug as defined under Section 201(p) of the Federal Food, Drug, and Cosmetic Act ("FFDCA"), and accordingly a New Drug Application ("NDA") approved by Food and Drug Administration

("FDA") pursuant to section 505(b) of the Act, was required before the drug product could be commercially marketed.

12. Photocure undertook the development of the product to establish, by adequate and well-controlled clinical trials, the product's safety and efficacy as a drug for treatment of actinic keratosis. Clinical studies on Metvixia™ (originally called "Metvix") began in 1997 outside the U.S. On February 24, 2000, Photocure filed an Investigational New Drug Application ("IND") with FDA for the Metvixia™ drug product.

13. On September 26, 2001, Photocure submitted an NDA for the product. FDA approved the NDA on July 27, 2004, permitting the commercial marketing of Metvixia™. The labeling approved by FDA describes Metvixia™ as an oil in water emulsion containing methylaminolevulinate hydrochloride at a concentration equivalent to 168 mg/g methylaminolevulinate. In combination with red light from a CureLight BroadBand Model CureLight 01 lamp, Metvixia™ is approved for use in treating non-hyperkeratotic actinic keratoses. On June 26, 2008, the FDA approved Metvixia™ in combination with red light from Aktilite® CL128, an LED-based narrow band lamp, for use in treating non-hyperkeratotic actinic keratoses.

14. The approved Metvixia™ drug product falls within claims 8 and 9 of the '267 patent and its approved use falls within claims 1 and 3-7 of the '267 patent.

THE AGENCY DECISION UNDER REVIEW

15. Section 156 of 35 U.S.C., added by the Drug Price Competition and Patent Term Restoration Act of 1984, permits the term of certain patents claiming approved drug products (or

their use or method of manufacture) to be extended to compensate for the length of time involved in obtaining regulatory review of the drug product. 35 U.S.C. § 156(a) provides that the term of a patent "shall be extended" if the following requirements are met:

> (1) the term of the patent has not expired before an application is submitted under subsection (d)(1) for its extension;
>
> (2) the term of the patent has never been extended under subsection (e)(1) of this section;
>
> (3) an application for extension is submitted by the owner of record of the patent or its agent and in accordance with the requirements of paragraphs (1) through (4) of subsection (d);
>
> (4) the product has been subject to a regulatory review period before its commercial marketing or use;
>
> (5)(A) * * * the permission for the commercial marketing or use of the product after such regulatory review period is the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred[.] 35 U.S.C. § 156(a).

Plaintiff's '267 patent satisfies each of these requirements. Defendants were therefore under a mandatory duty to extend the term of this patent in accordance with the provisions of 35 U.S.C. § 156.

16. On September 20, 2004, plaintiff timely filed an application in the PTO for a section 156 patent term extension for the '267 patent, which had issued on March 7, 2000 and is set to expire on March 8, 2016. The PTO issued its Notice of Final Determination denying plaintiff's application on April 11, 2007. Plaintiff timely filed a Request for Reconsideration of the PTO's decision on November 9, 2007. The PTO issued its Final Decision denying plaintiff's application on May 13, 2008. The PTO's denial of plaintiff's application constitutes final agency action on the application. *See* 37 C.F.R. § 1.750.

17. The PTO denied the application for a patent term extension for the '267 patent on the ground that it failed to satisfy the first commercial marketing requirement of 35 U.S.C.

§ 156(a)(5)(A). Even though Metvixia™ represents the first permitted commercial marketing or use of a drug containing methylaminolevulinate hydrochloride or a salt or ester of methylaminolevulinate hydrochloride as the active ingredient, the PTO reasoned that Metvixia™'s approval did not satisfy 35 U.S.C. § 156(a)(5)(A) because the term "product," as used in 35 U.S.C. § 156(a)(5)(A) and defined in 35 U.S.C. § 156(f), is properly construed, according to the PTO, to mean the "underlying molecule" exclusive of the "appended portions" that make the molecule an ester or salt. Final Decision at 5. Thus, the active ingredient of Metvixia™, according to the PTO, is aminolevulinic acid, and, since aminolevulinic acid hydrochloride previously was approved for commercial marketing, the approval of Metvixia™ did not represent the first permitted commercial marketing required by the statute.

COUNT I

18. Plaintiff repeats and incorporates by reference the allegations set forth in Paragraphs 1 through 17.

19. Defendants acted in a manner contrary to law in denying plaintiff's application for a patent term extension for the '267 patent in violation of 35 U.S.C. § 156, including 35 U.S.C. § 156(a)(5)(A).

20. The PTO's construction is contrary to law and will frustrate the overriding purpose of title II of the Drug Price Competition and Patent Term Restoration Act, which is to encourage research and innovation, including the development of new active ingredients.

COUNT II

21. Plaintiff repeats and incorporates by reference the allegations set forth in Paragraphs 1 through 20.

22. Defendants' denial of plaintiff's application for a patent term extension for the '267 patent is arbitrary and capricious and not in accordance with law, and therefore should be set aside under the Administrative Procedure Act, 5 U.S.C. §§ 701-706, including 5 U.S.C. § 706(2).

RELIEF REQUESTED

WHEREFORE, Plaintiff prays that the Court:

23. Issue a declaratory judgment that defendants acted unlawfully in denying plaintiff's application for patent term extension;

24. Issue a declaratory judgment that plaintiff's application for patent term extension satisfies each of the requirements set forth in 35 U.S.C. § 156;

25. Issue an order setting aside the defendant Commissioner's denial of plaintiff Photocure's application for patent term extension;

26. Issue an order compelling defendant Commissioner to comply with the requirements of 35 U.S.C. § 156(d)(2)(A) and, after compliance with those requirements, to take action to extend the term of U.S. Patent No.6,034,267 in accordance with the provisions of 35 U.S.C. § 156;

27. Award plaintiff its cost and reasonable attorneys' fees; and

28. Grant other or further relief as may be appropriate.

Respectfully submitted,

Date: July 11, 2008

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EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

MAY 13 2008

Kenyon & Kenyon
One Broadway
New York, NY 10004

In Re: Patent Term Extension
Application for
U.S. Patent No. 6,034,267

FINAL DECISION REGARDING PATENT TERM EXTENSION APPLICATION UNDER 35 U.S.C. § 156 FOR U.S. PATENT NO. 6,034,267

This is in response to the application for extension of the term of U.S. Patent No. 6,034,267 ("the '267 patent") filed under 35 U.S.C. § 156 in the United States Patent and Trademark Office ("USPTO") on September 22, 2004 ("the PTE Application"), and the Request for Reconsideration of Final Determination of Ineligibility for Patent Term Extension filed on November 13, 2007 ("the Request for Reconsideration"). The PTE Application was filed by PhotoCure ASA ("Applicant"), assignee and owner of the '267 patent. Extension was sought based upon the premarket review of METVIXIA™ (methyl aminolevulinate hydrochloride) under section 505(b) of the Federal Food Drug and Cosmetic Act ("FFDCA"). Because the Food and Drug Administration ("FDA") and the USPTO have determined that the approval of METVIXIA™ (methyl aminolevulinate hydrochloride) does not constitute the first permitted commercial marketing or use of the "product," the PTE Application is **DENIED** and the Request for Reconsideration is **DENIED**.

A. Factual Background

On July 27, 2004, the FDA approved NDA No. 21-415 for METVIXIA™ (methyl aminolevulinate hydrochloride).

On September 22, 2004, Applicant timely filed the PTE Application in the USPTO.

On November 7, 2006, the USPTO sent a letter to FDA, requesting the FDA's assistance in confirming that (1) the product identified in the PTE Application, METVIXIA™ (methyl aminolevulinate hydrochloride), was subject to a regulatory review period within the meaning of 35 U.S.C. § 156(g) before its first permitted commercial marketing or use and (2) the PTE application was filed within the sixty-day period beginning on the date the product received permission under the provision of law under which the applicable regulatory review period occurred for commercial marketing or use, as required by 35 U.S.C. § 156(d)(1). The November 7, 2006, letter notes at page 2 that "[a]minolevulinic acid hydrochloride had been previously approved by the FDA" and that "methyl aminolevulinate hydrochloride is an ester of aminolevulinic acid hydrochloride."

On March 5, 2007, FDA responded to the USPTO stating (1) FDA's approval of

METVIXIA™ (methyl aminolevulinate hydrochloride) does not represent the first permitted commercial marketing or use of the "product," as defined under 35 U.S.C. § 156(f)(1), and as interpreted by the courts, and (2) the PTE Application was timely filed.

On April 11, 2007, the USPTO mailed a Notice of Final Determination – Ineligible ("Notice") in which the USPTO states that the '267 patent is ineligible for patent term extension under 35 U.S.C. § 156. In particular, the Notice states:

> By the explicit terms of section 156(f)(2), the term "product" as it relates to a human drug product means the active ingredient of the new drug product. The active ingredient in the approved product METVIXIA™ is methyl aminolevulinate hydrochloride, which, as an ester of the previously-approved aminolevulinic acid hydrochloride, is by statute the same product as aminolevulinic acid hydrochloride. ... Furthermore, the prior approval of the active ingredient aminolevulinic acid hydrochloride in LEVULAN® by the Food and Drug Administration was under section 505 of the FFDCA, the same provision of law under which regulatory review of the product METVIXIA™ occurred.

On November 13, 2007, Applicant filed the Request for Reconsideration. The Request for Reconsideration states at page 3 that "the proper inquiry is simply, based on the plain language of the statute, whether or not the active ingredient in Levulan®, namely, aminolevulinic acid hydrochloride, is an ester (or the same as or a salt) of the active ingredient of Metvixia™." The Request for Reconsideration further states the following, at the paragraph bridging pages 3 and 4:

> the active ingredient of Metvixia™ is the hydrochloride salt of the ester methyl aminolevulinate, whereas the active ingredient of Levulan® is the hydrochloride salt of the acid aminolevulinic acid. Aminolevulinic acid hydrochloride is not the same as, or a salt or ester of, methyl aminolevulinate hydrochloride. The product methyl aminolevulinate hydrochloride therefore has not been previously approved because aminolevulinic acid hydrochloride does not "[fall] within the definition" of "product" as that term is properly construed. *See* [*Glaxo Operations UK, Ltd v. Quigg*, 894 F.2d 392, 394 (Fed. Cir. 1990)]. It therefore follows that Metvixia™ is not precluded from patent term extension eligibility by the previous approval of aminolevulinic acid hydrochloride.

(Emphasis in the original). The Request for Reconsideration also states at page 5 that "there are substantial differences between methyl aminolevulinate hydrochloride and [5-aminolevulinic acid ("ALA")] hydrochloride, as evidenced by the attached Declaration of Dr. Kristian Berg in Support of Grant of Patent Term Extension with Respect to U.S. Patent No. 6,034,267 and accompanying exhibits."

B. Decision

1. The Plain Language of 35 U.S.C. § 156(f) Shows That METVIXIA™ (methyl aminolevulinate hydrochloride) Is Not the First Permitted Commercial Marketing or Use of the "Product" As Required by 35 U.S.C. § 156(a)(5)(A)

Section 156(a) of Title 35 sets forth several requirements that must be met before the Director can extend the term of a patent. *See* 35 U.S.C. §§ 156 (a)(1)-(a)(5), (d)(1), & (e)(1). Section 156(a)(5)(A) requires that:

> the permission for the commercial marketing or use of the product ... [be] the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred.

(Emphasis added). The term "product" as used in section 156(a)(5)(A) is defined in section 156(f)(1) as a "drug product," and the term "drug product" is defined in section 156(f)(2) as the "active ingredient of [a] new drug, antibiotic drug, or human biological product ... including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient." 35 U.S.C. § 156(f) (emphasis added). Hence, by the explicit terms of section 156(f)(2), the term "product" as used in section 156 includes: (i) a non-salified and non-esterified form of a molecule (*i.e.*, the "active ingredient"); (ii) any salt of the molecule (*i.e.*, the "salt ... of the active ingredient"); and (iii) any ester of the molecule (*i.e.*, the " ... ester of the active ingredient").[1] Because a "product" includes all three forms, any salt of a molecule is statutorily the same "product" as any ester of the molecule for purposes of the patent term extension provisions in section 156. Further, the plain meaning of the phrase "any ester" encompasses any ester, including salified and non-salified esters.

Prior to the approval of METVIXIA™ (methyl aminolevulinate hydrochloride), the FDA approved LEVULAN® (aminolevulinic acid hydrochloride). There is no dispute that ALA is present in both METVIXIA™ and LEVULAN® as the underlying molecule. For example, at page 2 of the Declaration attached to the Request for Reconsideration, Dr. Berg states that METVIXIA™ "has as its active ingredient the hydrochloride salt of the methyl ester of ALA," and that LEVULAN® "has the hydrochloride salt of ALA as its active ingredient." Consequently, the approved "product" is the same for both METVIXIA™ and LEVULAN® under section 156, *i.e.*, ALA merely formulated differently in each product. The later approved METVIXIA™ (methyl aminolevulinate hydrochloride) thus does not represent the first permitted commercial marketing or use of the "product" under the provision of law under which such regulatory review occurred. The USPTO therefore concludes that the PTE Application does not

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[1]The plain language of section 156(f) makes clear that the same definition of "product" is to be applied throughout section 156. Section 156(f) explicitly states that its provisions are "for purposes of this section." Thus, the term "product" as used throughout 35 U.S.C. § 156—for eligibility under section 156(a) and for enforcement under section 156(b)—has but one meaning.

satisfy the requirement of section 156(a)(5)(A) and the '267 patent is ineligible for a patent term extension. Accordingly, the PTE Application must be **DENIED**.

2. Judicial Precedent Confirms That METVIXIA™ (methyl aminolevulinate hydrochloride) Is Not the First Permitted Commercial Marketing or Use of the "Product" As Required by 35 U.S.C. § 156(a)(5)(A)

Judicial precedent confirms that the USPTO's application of the definition of "product," as that term is used in section 156(a)(5)(A), is correct. In *Fisons v. Quigg*, 1988 WL 150851 (D.D.C. 1988) ("*Fisons I*"), the district court addressed the meaning of the term "product." The district court considered both the plain language of section 156(a)(5)(A) and its legislative history. With respect to the latter, the district court observed:

> Upon examination, the specific purpose of Section 156(a)(5)(A) appears to have been relatively narrow—to restore lost patent life only for "pioneer" drugs. A report by the Congressional Office of Technology Assessment ("OTA") to the 97th Congress provided the factual foundation for the restriction of patent restoration benefits to new chemical entities. The OTA report stated: "Although important pharmaceutical innovations may result from new therapeutic applications of existing chemicals ... many of the pharmaceutical breakthroughs that have occurred have resulted from NCE (new chemical entity) research and the development of NCEs generally has required more time and money than other types of innovation and has involved greater risks." The House Committee on Energy and Commerce explained that the bill "requires extensions to be based on the first approval of the product because the only evidence available to Congress showing that patent time has been lost is data on so-called class I, new chemical entity drugs."

Fisons I, 1988 WL 150851 at *7. After making these observations, the district court found that "Congress's intent was to restore patent life only to new chemical entities." The district court thus construed section 156(a)(5)(A) in a straightforward way:

> In the definitional provision of Section 156, the term "product" is defined as a "human drug product." 35 U.S.C. § 156(f)(1)(A). This term is further defined in the next subparagraph as "the *active ingredient* of a new drug, antibiotic drug, or human biological product ... including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient." 35 U.S.C. § 156(f)(2) (emphasis added in original). Substituting this definition directly back into Section 156(a)(5)(A) yields the statement that a patent is ineligible for extension if it is not the first permitted commercial marketing or use of the active ingredient contained in that approved patented product.

Id. at *5.

The Federal Circuit affirmed the district court's interpretation. *See Fisons v. Quigg*, 876 F.2d 99 (Fed. Cir. 1989) ("*Fisons II*"). The Federal Circuit stated: "In sum, we hold that the district court correctly applied the definition given in 35 U.S.C. § 156(f) to the term 'product' used in section 156(a)(5)(A). We are convinced that such an interpretation comports with the intent of Congress as expressed in the statute." *Fisons II*, 876 F.2d at 102.

The Federal Circuit later interpreted the term "active ingredient" in *Pfizer, Inc. v. Dr. Reddy's Labs.*, Ltd., 359 F.3d 1361 (Fed. Cir, 2004). There, the Federal Circuit accepted the FDA's definition of the term "active ingredient" as meaning "active moiety." *See id.* at 1366 (citing Abbreviated New Drug Application Regulations: Patent and Exclusivity Provisions, 59 Fed. Reg. 50,338, 50,358 (F.D.A. Oct. 3, 1994)). It likewise accepted that "active moiety" means "the molecule or ion excluding those appended portions of the molecule that cause the drug to be an ester, salt ... responsible for the physiological or pharmacological action of the drug substance," based upon the FDA's regulations. *Id.* (quoting 21 C.F.R. § 314.108(a)) (omission in original). Hence, the Federal Circuit has construed the term "active ingredient" as used in section 156(f)(2) to mean the underlying molecule, *i.e.*, the molecule or ion responsible for the physiological or pharmacological action of the drug, excluding those appended portions of the molecule that cause the drug to be an ester or salt.

Substituting this definition for the word "active ingredient" as it appears in section 156, the term "drug product" in section 156(f)(2) must mean the underlying molecule as well as any salt or ester of the underlying molecule, since it is defined as "active ingredient ... including any salt or ester of the active ingredient." Further, because "product" is defined as "drug product" in section 156(f)(1)(A), "product" likewise must mean the underlying molecule as well as any salt or ester of the underlying molecule. That definition conforms with the plain language of section 156(f). What is more, the Federal Circuit confirmed in *Pfizer* that only the first approval for any given "active ingredient" can trigger a patent term extension under 35 U.S.C. § 156, regardless of whether that first approval was for an underlying molecule, a salt of the underlying molecule, or an ester of the underlying molecule. *See Pfizer*, 359 F.3d at 1366 ("The statute [referring to 35 U.S.C. § 156] foresaw variation in the salt or ester of an active ingredient, and guarded against the very loophole now urged.").

Here, before approving METVIXIA™ (methyl aminolevulinate hydrochloride) in 2004, the FDA approved LEVULAN® (aminolevulinic acid hydrochloride) in 1999. As explained above, ALA is the underlying molecule in both METVIXIA™ and LEVULAN®. ALA is simply formulated differently in the two different drugs: as a hydrochloride salt of its methyl ester in METVIXIA™, and as a hydrochloride salt in LEVULAN®. However, the difference in formulation does not matter for purposes of defining a product in section 156. The statutory definition of "product" includes the underlying molecule as well as any salt or ester of the underlying molecule. Accordingly, METVIXIA™ (methyl aminolevulinate hydrochloride) is not the first permitted commercial marketing or use of the "product" as required by 35 U.S.C. § 156(a)(5)(A) because of the earlier approval of LEVULAN® (aminolevulinic acid hydrochloride).

Finally, the FDA has issued a regulation defining the term "active ingredient" of a pharmaceutical "product" for purposes of patent term extension under 35 U.S.C. § 156. Specifically, 21 C.F.R. § 60.1(a) states that "[t]his part [referring to Part 60] sets forth procedures and requirements for the [FDA]'s review of applications for the extension of the term of certain patents under 35 U.S.C. § 156." That provision further states that "[FDA] actions in this area include [*inter alia*] [a]ssisting the [USPTO] in determining eligibility for patent term restoration." 21 C.F.R. § 60.1(a)(1). Section 60.3 then provides a series of definitions to be used in Part 60 in addition to the definitions already contained in 35 U.S.C. § 156. 37 C.F.R. § 60(b)(2) defines "active ingredient" for purposes of a patent extension to mean a drug's active moiety, *i.e.*, its therapeutically active component. It states:

> Active ingredient means any component that is intended to furnish pharmacological activity or other direct effects in the diagnosis, cure, mitigation, treatment or prevention of disease, or to affect the structure or function of the body of man or of animals. The term includes those components that may undergo chemical change in the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect.

21 C.F.R. § 60.3 (b)(2). Applying the FDA's regulations in this case, ALA is the "active ingredient" of not just LEVULAN® (aminolevulinic acid hydrochloride), but also of METVIXIA™ (methyl aminolevulinate hydrochloride); it is simply formulated as a hydrochloride salt of its methyl ester in METVIXIA™, and as a hydrochloride salt in LEVULAN®.

The USPTO recognizes that *Glaxo* also concerns section 156(f). However, the USPTO observes that *Glaxo* is factually distinguishable because the Federal Circuit did not address the definition of "active ingredient" in that case. Rather, the Federal Circuit focused on the USPTO's argument that the term "product" did not have the literal meaning set forth in section 156(f)(2), but instead meant "any 'new chemical entity,' *i.e.*, 'new active moiety.'" Rejecting that argument, the Federal Circuit explained that Congress provided a definition of the term "product" in section 156(f)(2) and that Congress "selected terms with narrow meanings that it chose from among many alternatives." *Glaxo*, 894 F.2d at 399 (footnoting as examples of other possible words "new molecular entity," "active moiety," and "new chemical entity"). The Federal Circuit did not discuss the definition of the term "active ingredient" because, unlike here, the determination of the active ingredient was not in dispute in *Glaxo*.

The most that can be said about *Glaxo* is that the Federal Circuit acknowledged that the term "product" was not expressly defined by Congress to mean "active moiety," since those words do not appear in section 156(f)(2). However, *Glaxo* does not hold that the term "active ingredient" as used in section 156(f)(2) does not mean "active moiety." In fact, the Federal Circuit later accorded the term "active ingredient" with that precise definition in *Pfizer*. *See Pfizer*, 359 F.3d at 1366. Accordingly, the USPTO's determination that the '267 patent is

ineligible for extension pursuant to section 156 is supported by, and consistent with, *Glaxo*. As such, the PTE Application must be **DENIED**.

3. Applicant's Argument That METVIXIA™ Is Eligible for Patent Term Extension Because Neither Methyl Aminolevulinate Hydrochloride nor Any Salt or Ester of Methyl Aminolevulinate Hydrochloride Has Been Previously Approved for Commercial Marketing or Use Is Unpersuasive

Applicant states at page 3 of the Request for Reconsideration that "the proper inquiry is simply, based on the plain language of the statute, whether or not the active ingredient in Levulan®, namely, aminolevulinic acid hydrochloride, is an ester (or the same as or a salt) of the active ingredient of Metvixia™." At the paragraph bridging pages 3 and 4 of the Request for Reconsideration, Applicant concludes that the '267 patent is eligible for extension, because "[a]minolevulinic acid hydrochloride is not the same as, or a salt or ester of, methyl aminolevulinate hydrochloride."

In making the above statements in the Request for Reconsideration, Applicant ignores both (i) the full scope of the relationship between aminolevulinic acid hydrochloride and methyl aminolevulinate hydrochloride, and (ii) the Federal Circuit's decision in *Pfizer* that the term "active ingredient," when properly construed, means the underlying molecule, *i.e.*, the molecule or ion responsible for the physiological or pharmacological action of the drug, excluding those appended portions of the molecule that cause the drug to be an ester or salt. Applying the Federal Circuit's construction of the term "active ingredient" in *Pfizer* to the present case, ALA is the "active ingredient" of both METVIXIA™ and LEVULAN®. Consequently, the active ingredient in METVIXIA™ - ALA formulated as a hydrochloride salt of its methyl ester - has already been approved by the FDA with the approval of LEVULAN® (ALA formulated as a hydrochloride salt). Applicant's statement that neither methyl aminolevulinate hydrochloride nor an ester or salt of methyl aminolevulinate hydrochloride had previously been approved, while correct, is irrelevant to the calculus here. The USPTO must therefore conclude that the PTE Application does not satisfy the requirement of section 156(a)(5)(A) and the '267 patent is ineligible for a patent term extension. Accordingly, the PTE Application must be **DENIED**.

4. Applicant's Argument That There Are Substantial Differences Between Methyl Aminolevulinate Hydrochloride and Aminolevulinic Acid Hydrochloride Is Unpersuasive

Applicant states the following at page 5 of the Request for Reconsideration:

> there are substantial differences between methyl aminolevulinate hydrochloride and ALA hydrochloride, as evidenced by the attached Declaration of Dr. Kristian Berg in Support of Grant of Patent Term Extension with Respect to U.S. Patent

No. 6,034,267 and accompanying exhibits. These include substantial differences in selectivity of uptake by target lesions, penetration of target lesions, (unwanted) systemic distribution, pain resulting from use in PDT, and mechanisms of cell uptake. Accordingly, methyl aminolevulinate hydrochloride should not be considered the same "product" as aminolevulinic acid hydrochloride (regardless of how "product" is construed).

The existence of "substantial differences" between methyl aminolevulinate hydrochloride and aminolevulinic acid hydrochloride, even if verified, has no bearing on whether the PTE Application satisfies the requirement of section 156(a)(5)(A). For the reasons stated in the analysis above, the approved "product" is the same for both METVIXIA™ and LEVULAN® under section 156, *i.e.*, ALA merely formulated differently in each product. Nothing in the statutory language of 35 U.S.C. § 156 or in judicial precedent considering section 156 creates a "substantial differences" exception in the inquiry of whether the requirement of section 156(a)(5)(A) has been satisfied. For the reasons stated earlier herein, the USPTO concludes that the PTE Application does not satisfy the requirement of section 156(a)(5)(A) and the '267 patent is ineligible for a patent term extension. Therefore, the PTE Application must be **DENIED**.

5. Conclusion

For the reasons stated above, Applicant's request for extension of the patent term of the '267 patent is **DENIED**, and Applicant's Request for Reconsideration is **DENIED**.

This is considered a final agency decision.

Any correspondence with respect to this matter should be addressed as follows:

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Telephone inquiries related to this determination should be directed to Raul Tamayo at (571) 272-7728.

Robert A. Clarke
Director
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Office of Regulatory Policy
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 51, Rm. 6222
Silver Spring, MD 20993-0002

RE: METVIXIA™ (methyl aminolevulinate hydrochloride)
FDA Docket No.: 2007E-0001

Attention: Beverly Friedman

EXHIBIT B

US006034267A

United States Patent [19]

Gierskcky et al.

[11] Patent Number: 6,034,267

[45] Date of Patent: Mar. 7, 2000

[54] **ESTERS OF 5-AMINOLEVULINIC ACID AS PHOTOSENSITIZING AGENTS IN PHOTOCHEMOTHERAPY**

[75] Inventors: **Karl E. Gierskcky; Johan Moan; Qian Peng; Harald Steen; Trond Warloe; Alf Bjorseth,** all of Oslo, Norway

[73] Assignee: **PhotoCure AS,** Olso, Norway

[21] Appl. No.: 08/913,257

[22] PCT Filed: Mar. 8, 1996

[86] PCT No.: PCT/GB96/00553

§ 371 Date: Dec. 5, 1997

§ 102(e) Date: Dec. 5, 1997

[87] PCT Pub. No.: WO96/28412

PCT Pub. Date: Sep. 19, 1996

[30] Foreign Application Priority Data

Mar. 10, 1995 [GB] United Kingdom 9504948
Dec. 18, 1995 [GB] United Kingdom 9525822

[51] Int. Cl.[7] C07C 229/00; A61K 31/195

[52] U.S. Cl. 560/155; 514/506; 436/74; 436/63; 436/64; 436/96; 435/29; 435/34; 424/9.6

[58] Field of Search 560/155; 514/506

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Primary Examiner—Gary Geist
Assistant Examiner—Robert Deemie
Attorney, Agent, or Firm—Schwegman, Lundberg, Woessner & Kluth, P.A.

[57] **ABSTRACT**

The invention provides a pharmaceutical compositions comprising a compound of formula I:

$$R_2{}^2N—CH_2COCH_2—CH_2CO—OR^1 \quad (I)$$

wherein, R^1 and R^2 have any of the values defined in the specification; or a salt thereof; and a pharmaceutically acceptable carrier or excipient. The invention also provides a method for the diagnosis or photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body using such compounds or salts.

16 Claims, 23 Drawing Sheets

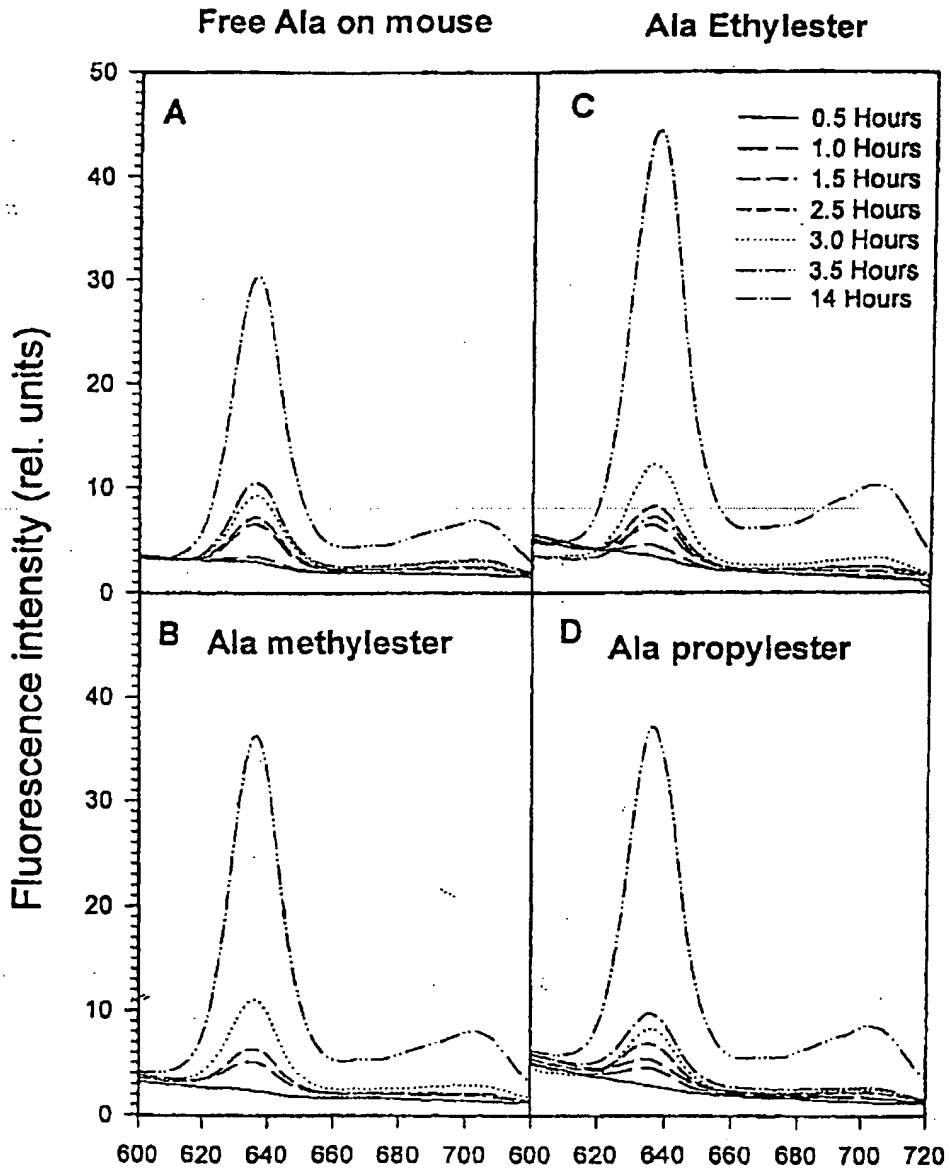

Free Ala on mouse
Ala Ethylester
A
C
B Ala methylester
D Ala propylester
0.5 Hours
1.0 Hours
1.5 Hours
2.5 Hours
3.0 Hours
3.5 Hours
14 Hours
Fluorescence intensity (rel. units)
0
10
20
30
40
50
600 620 640 660 680 700 600 620 640 660 680 700 720
Wavelength (nm)

FIG. 1.

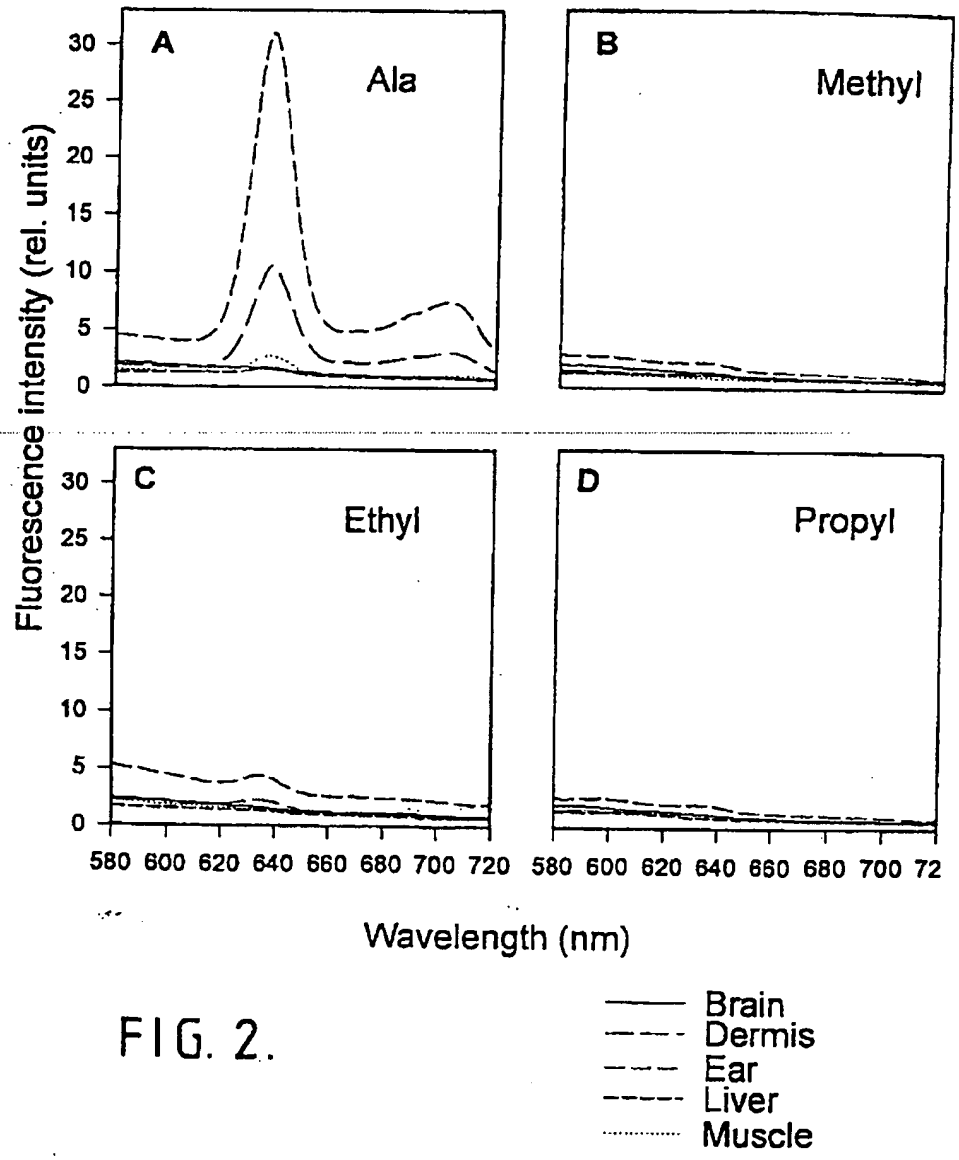

A
Ala
B
Methyl
C
Ethyl
D
Propyl
Fluorescence intensity (rel. units)
0
5
10
15
20
25
30
580 600 620 640 660 680 700 720
Wavelength (nm)
Brain
Dermis
Ear
Liver
Muscle

FIG. 2.

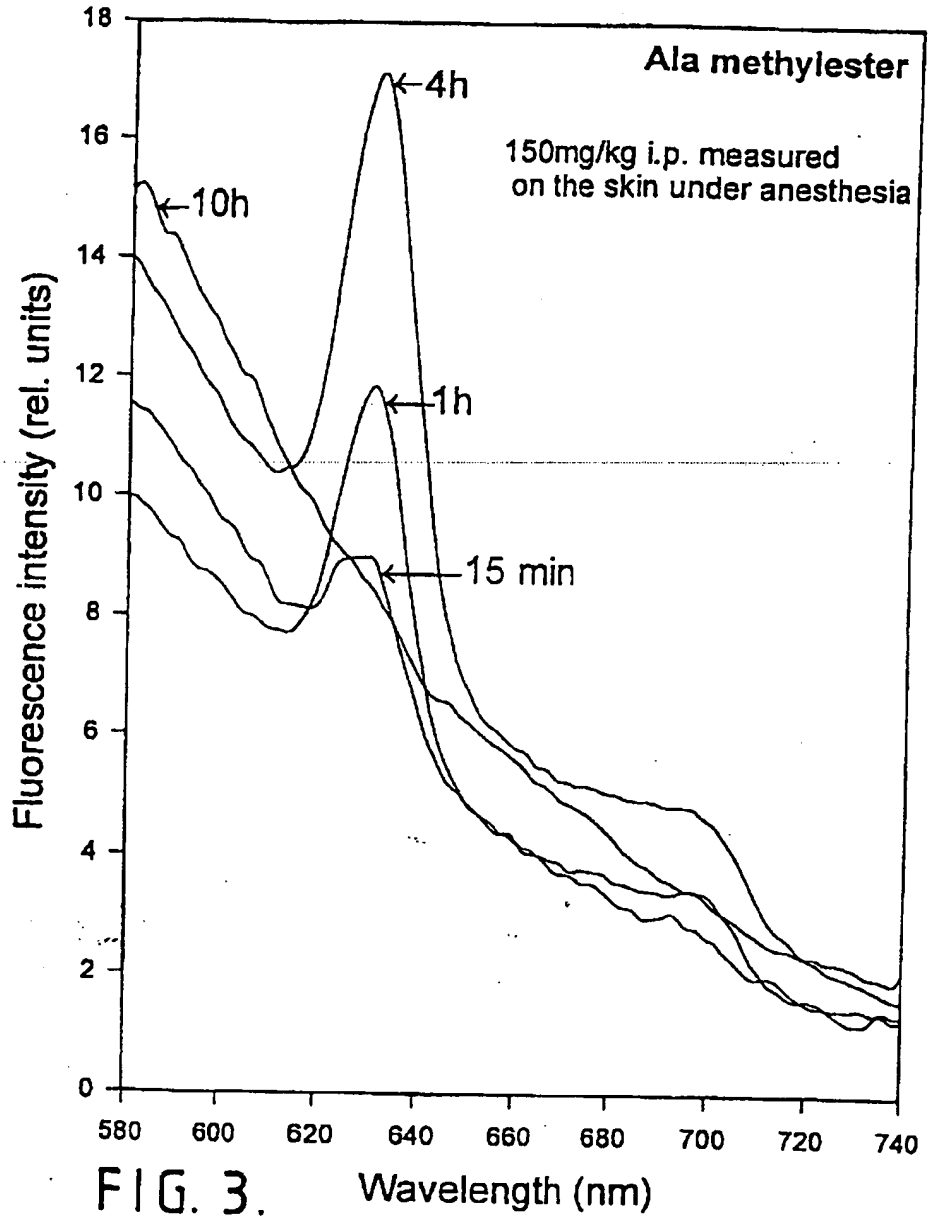

Ala methylester
150mg/kg i.p. measured
on the skin under anesthesia
4h
10h
1h
15 min
Fluorescence intensity (rel. units)
Wavelength (nm)
0
2
4
6
8
10
12
14
16
18
580
600
620
640
660
680
700
720
740

FIG. 3.

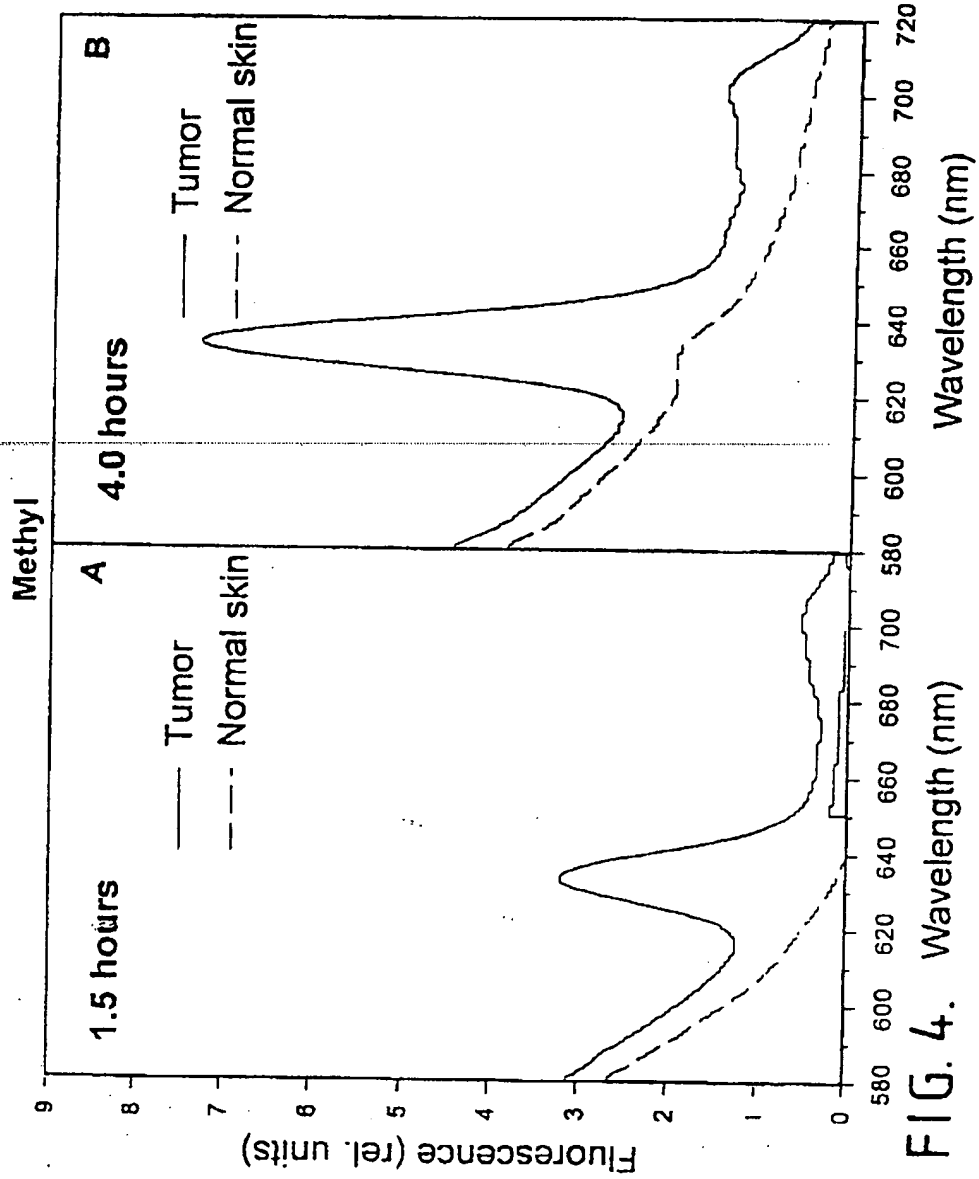

Methyl
A
1.5 hours
Tumor
Normal skin
B
4.0 hours
Tumor
Normal skin
Fluorescence (rel. units)
Wavelength (nm)
Wavelength (nm)

FIG. 4.

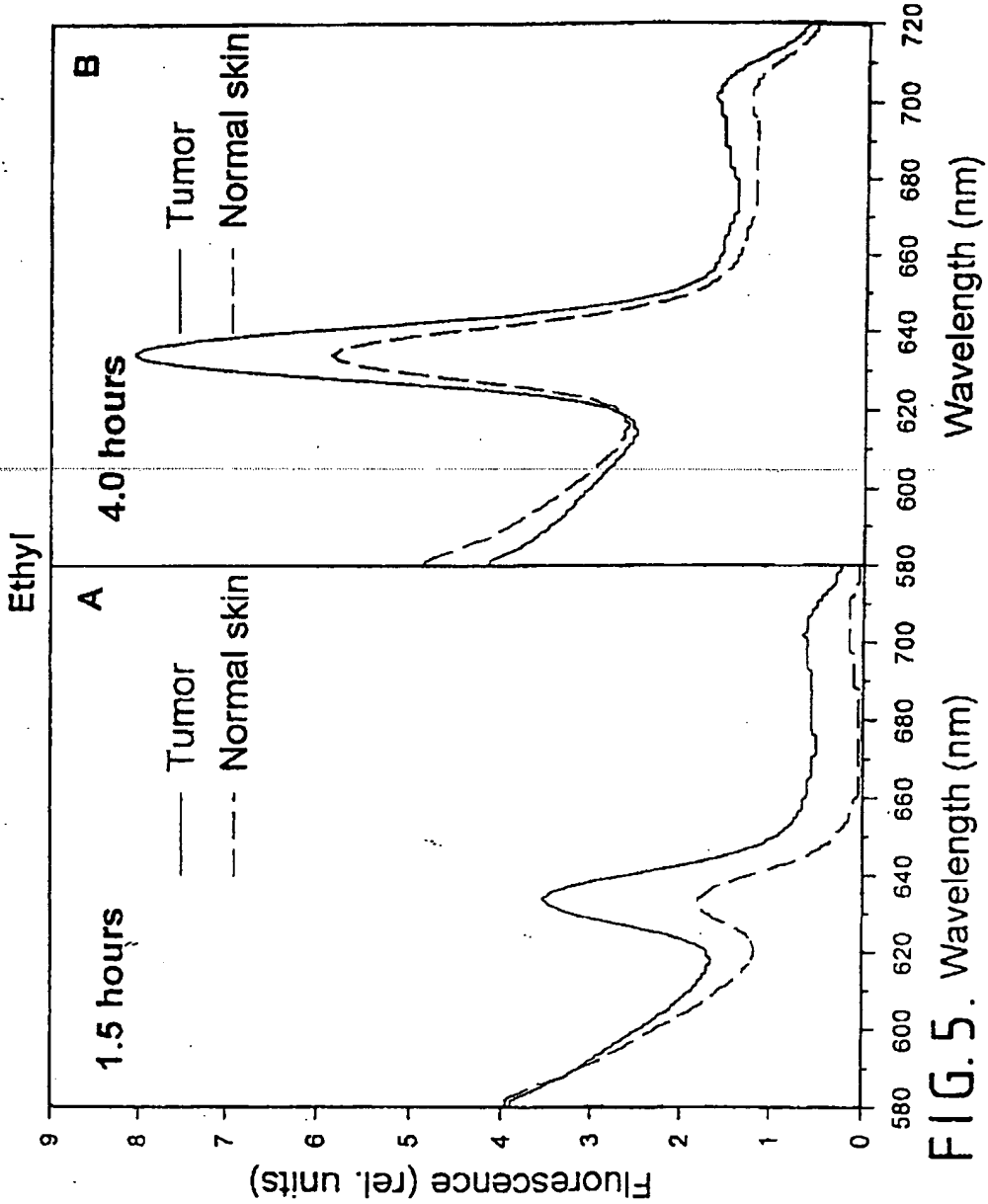

Ethyl
A
1.5 hours
Tumor
Normal skin
B
4.0 hours
Tumor
Normal skin
Fluorescence (rel. units)
0
1
2
3
4
5
6
7
8
9
580
600
620
640
660
680
700
720
Wavelength (nm)
Wavelength (nm)

FIG. 5.

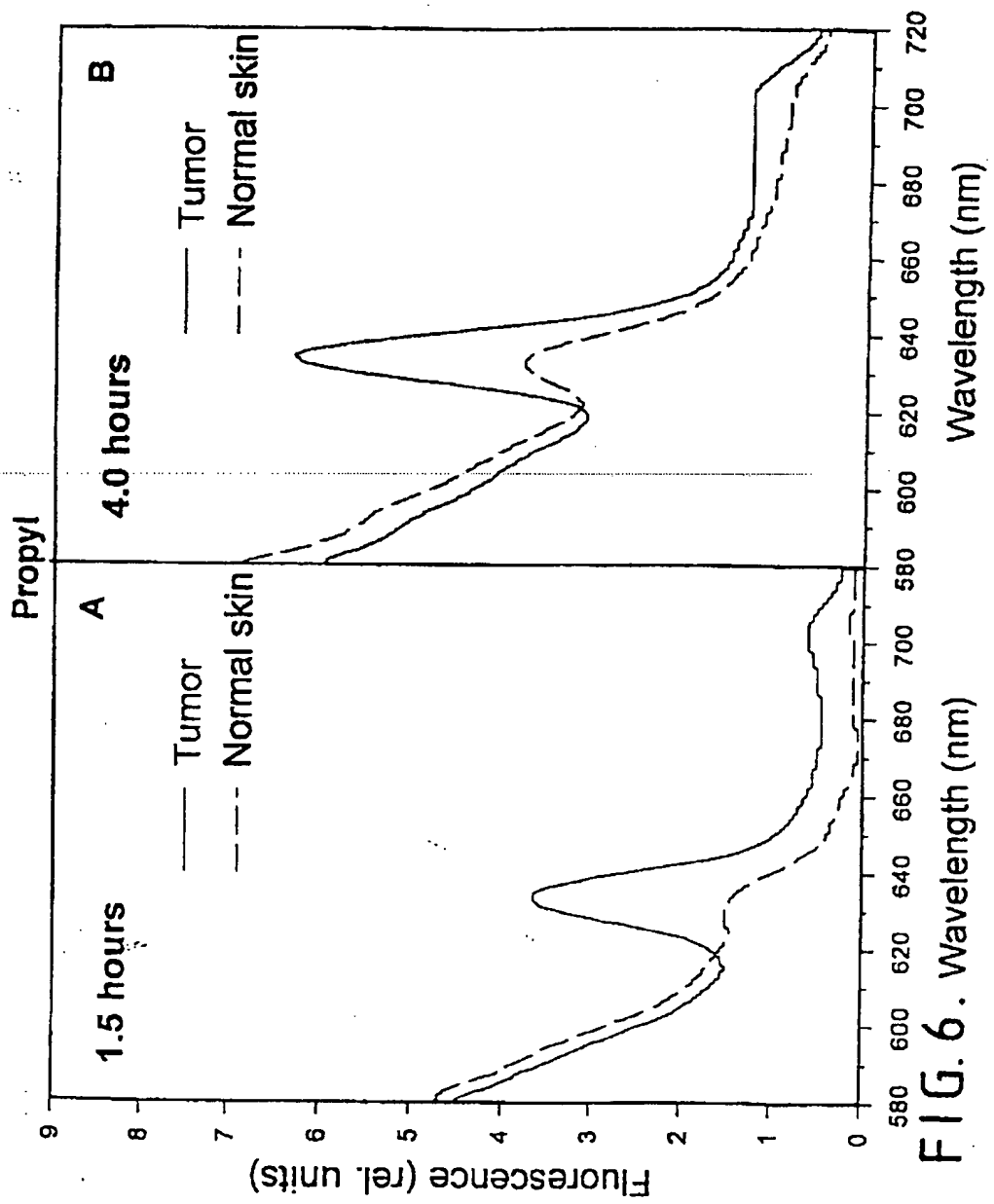

Propyl
A
1.5 hours
Tumor
Normal skin
B
4.0 hours
Tumor
Normal skin
Fluorescence (rel. units)
0
1
2
3
4
5
6
7
8
9
580
600
620
640
660
680
700
720
Wavelength (nm)
Wavelength (nm)

FIG. 6.

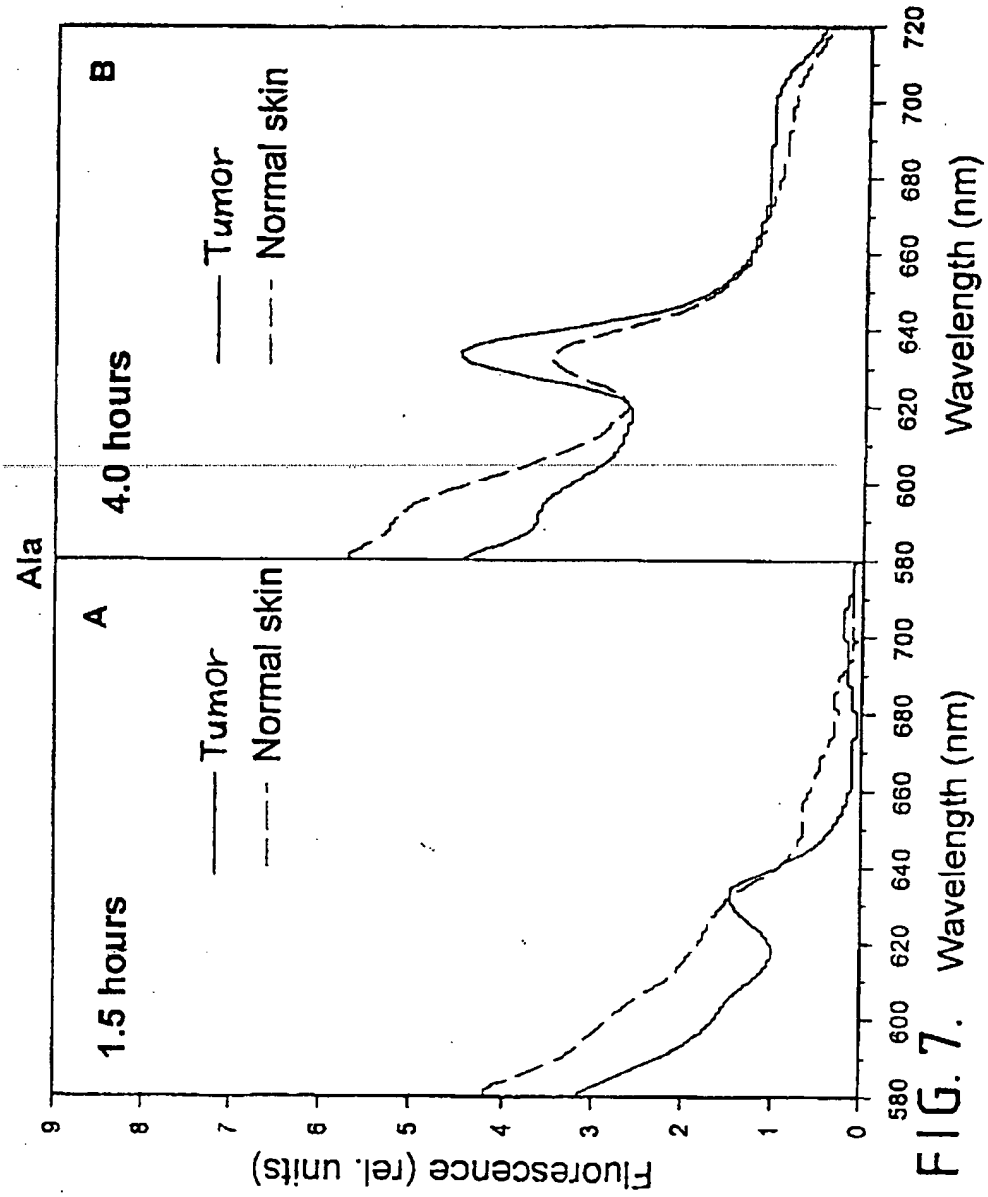
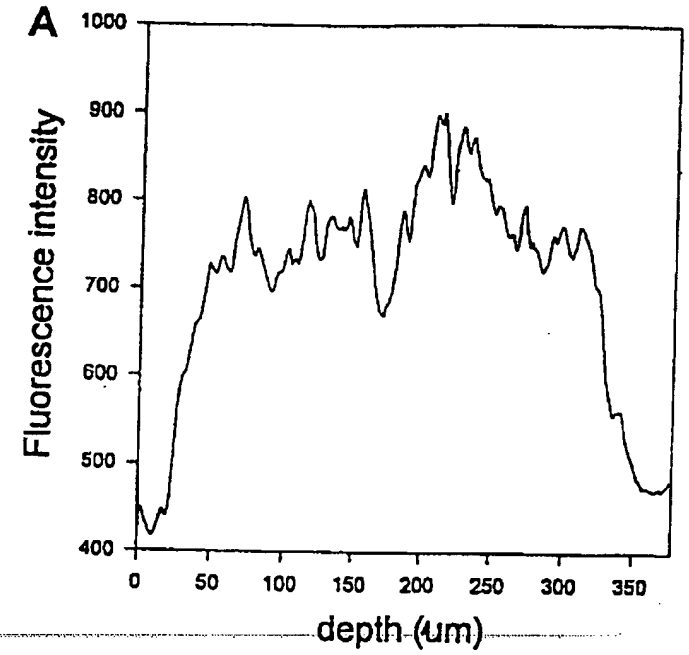
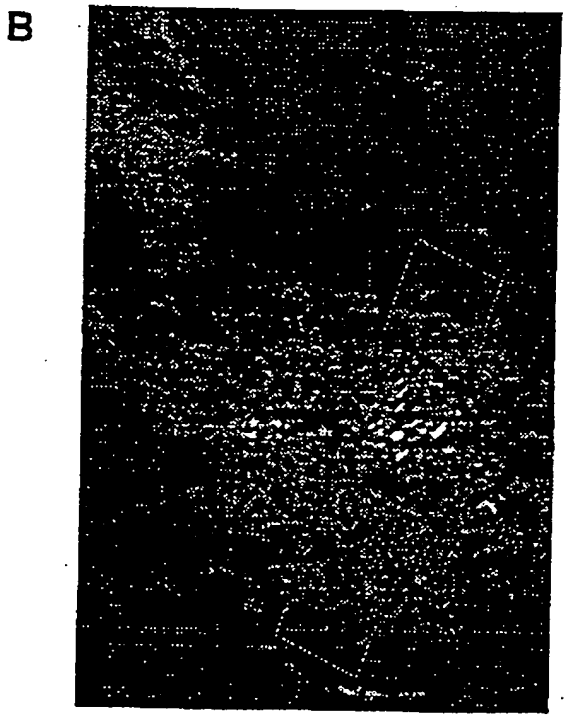

Ala
A
1.5 hours
Tumor
Normal skin
B
4.0 hours
Tumor
Normal skin
Fluorescence (rel. units)
0
1
2
3
4
5
6
7
8
9
580
600
620
640
660
680
700
720
Wavelength (nm)
Wavelength (nm)

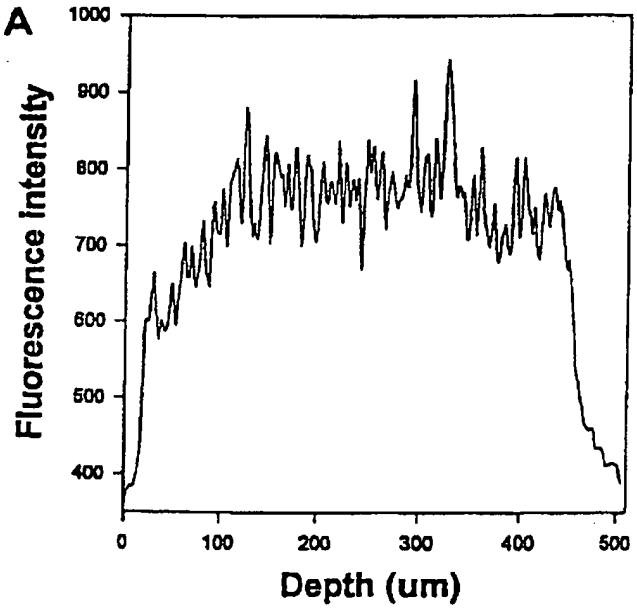
FIG. 7.

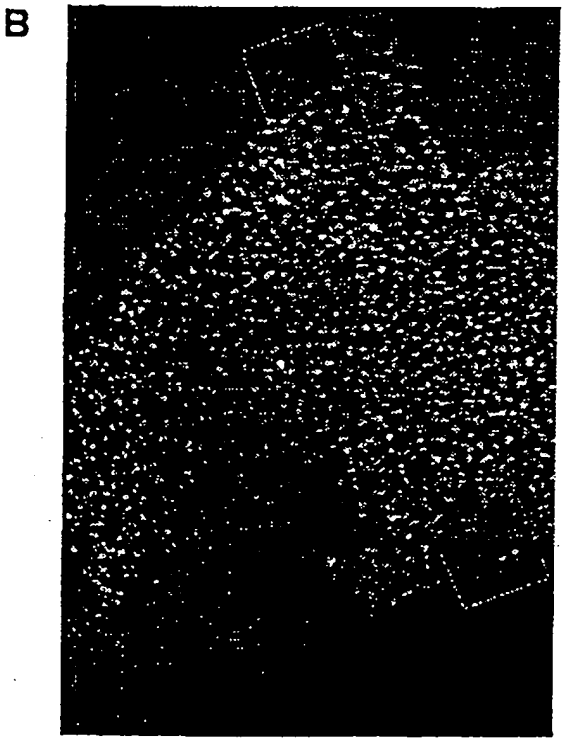




Free ALA

Figure 8





ALA methylester

Figure 9

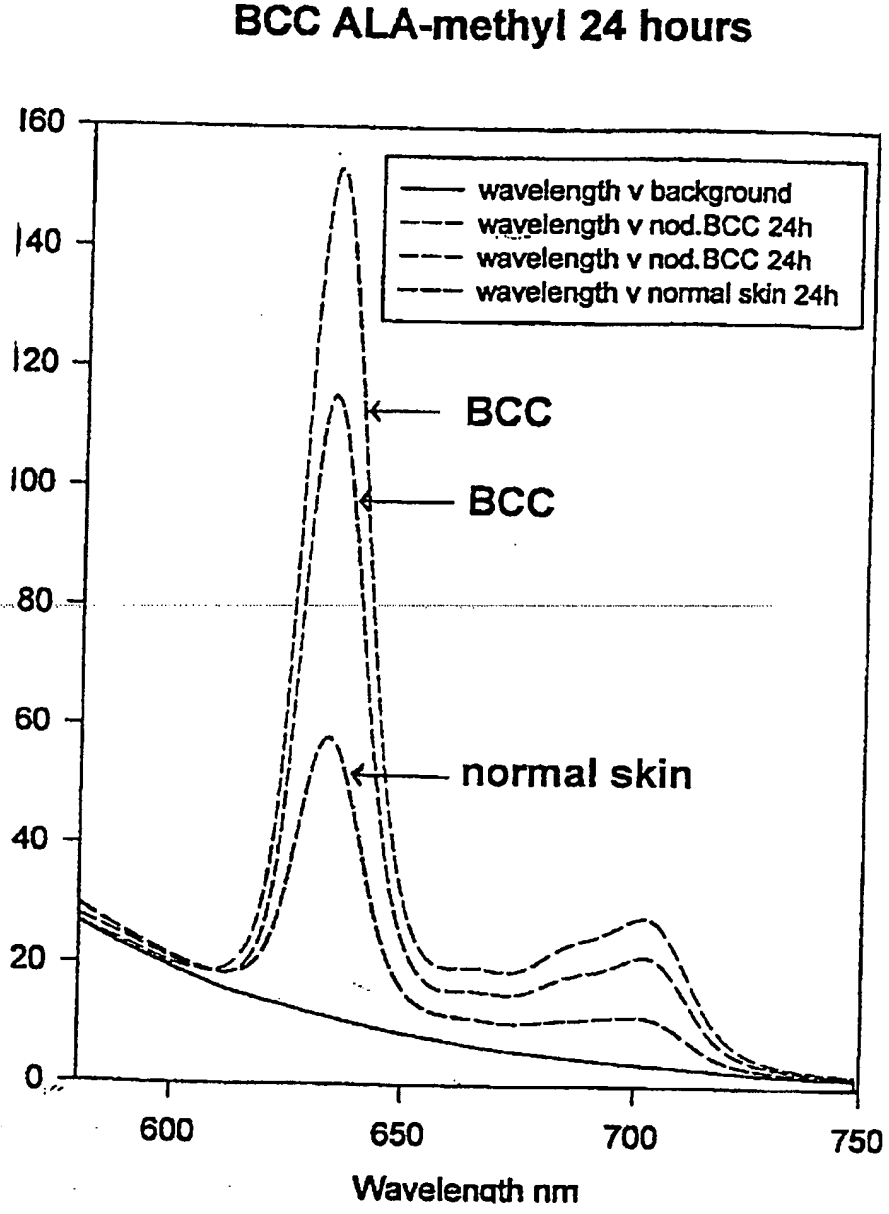

BCC ALA-methyl 24 hours
160
140
120
100
80
60
40
20
0
600
650
700
750
Wavelength nm
wavelength v background
wavelength v nod.BCC 24h
wavelength v nod.BCC 24h
wavelength v normal skin 24h
BCC
BCC
normal skin

Figure 10

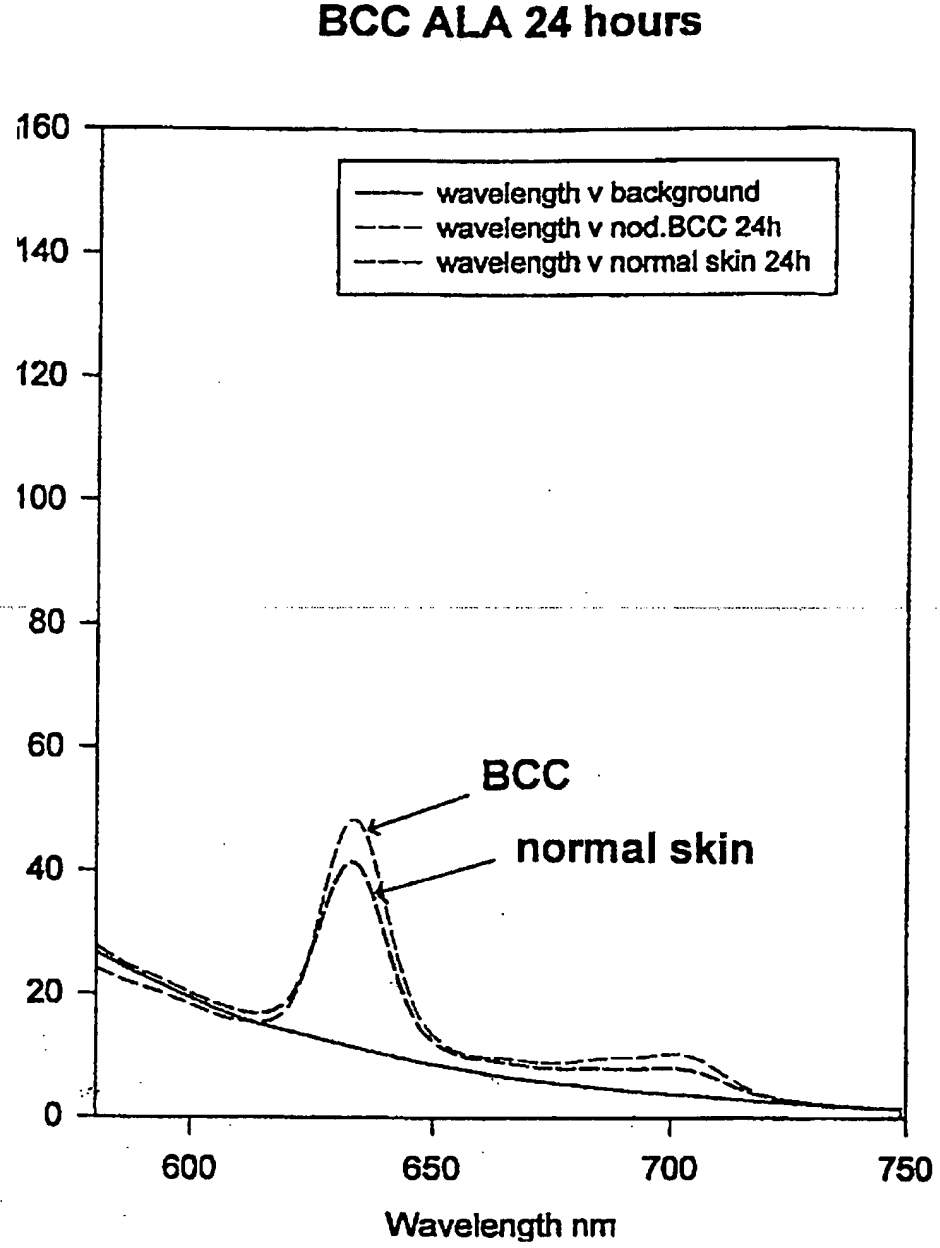
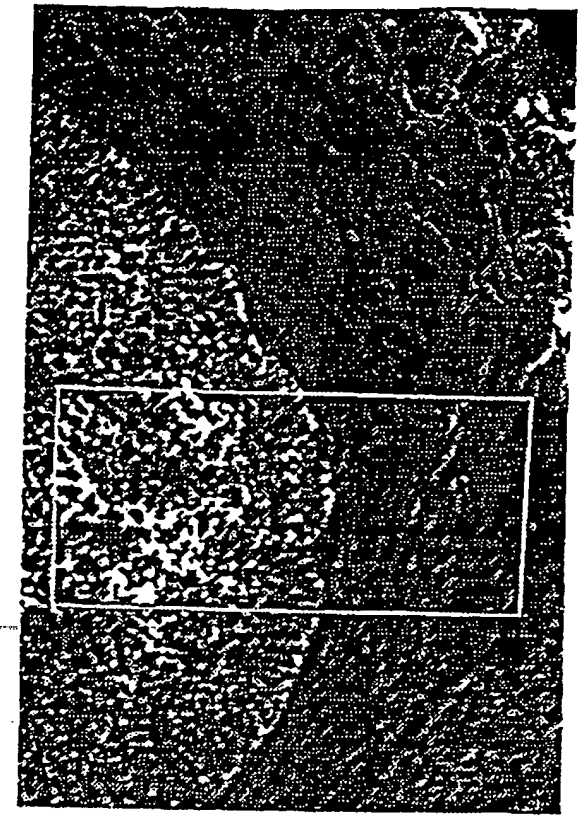

BCC ALA 24 hours
wavelength v background
wavelength v nod.BCC 24h
wavelength v normal skin 24h
160
140
120
100
80
60
40
20
0
BCC
normal skin
600
650
700
750
Wavelength nm

Figure 11

B



A

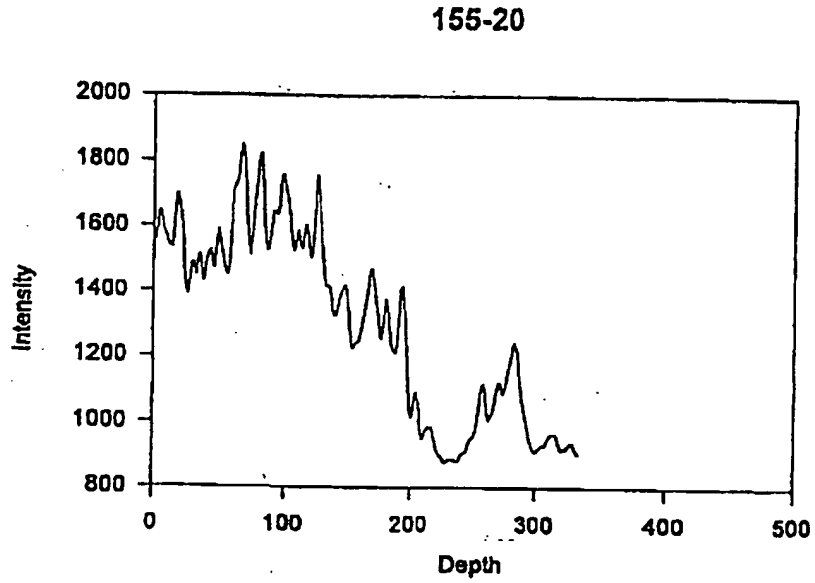
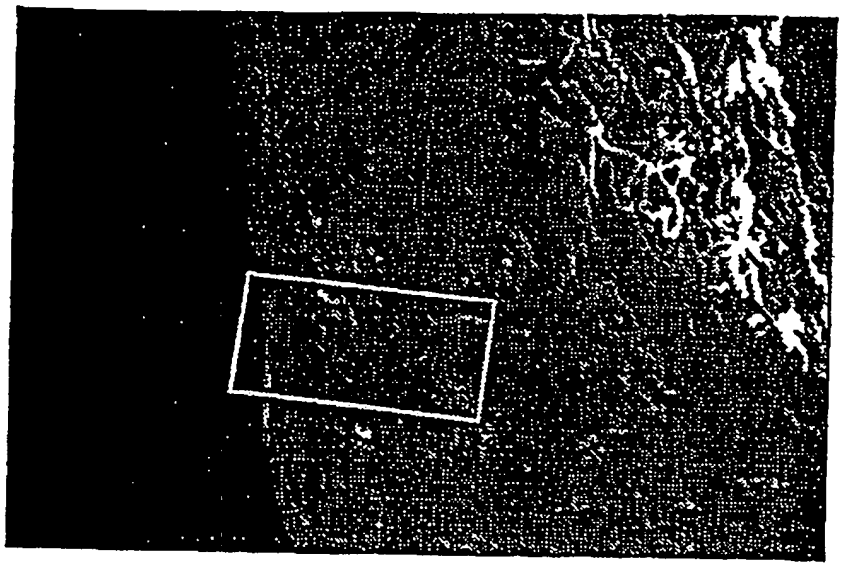

155-20
2000
1800
1600
1400
1200
1000
800
Intensity
0
100
200
300
400
500
Depth

Figure 12

B



156-1

A

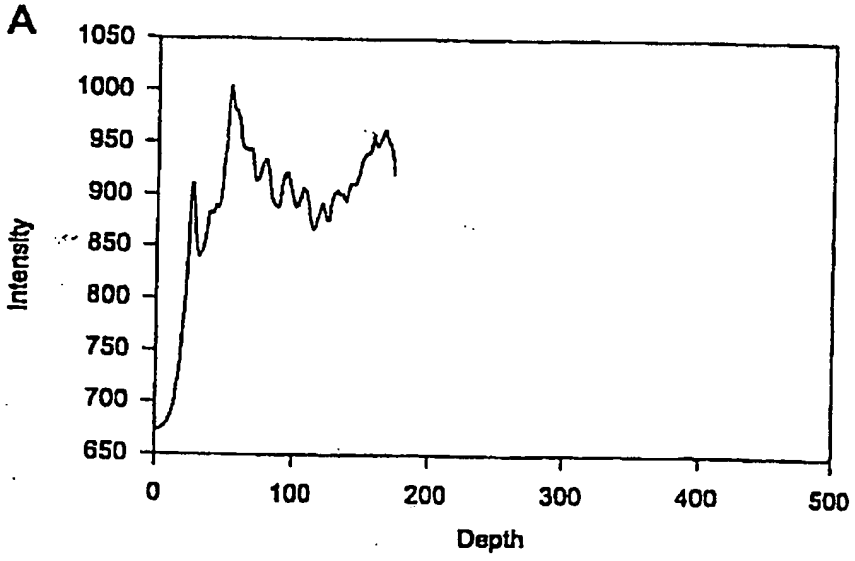
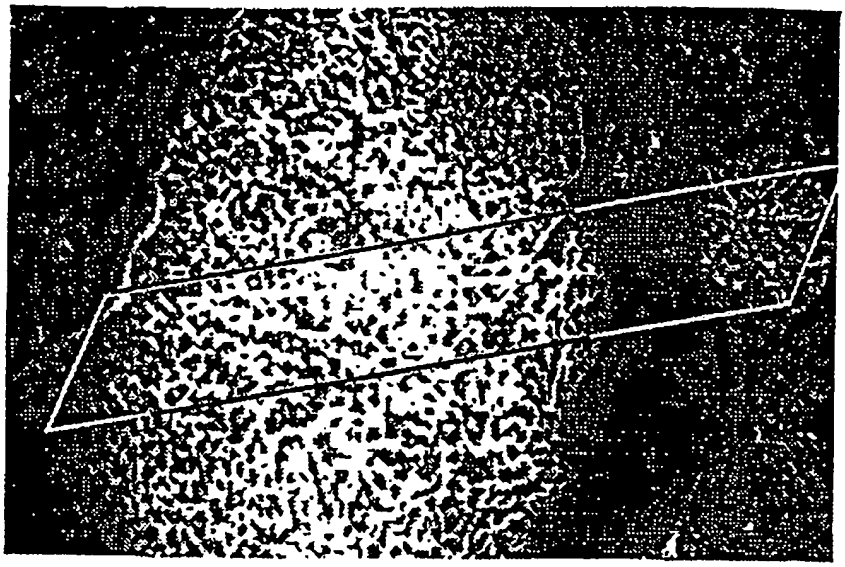


Figure 13

B



153-11

A

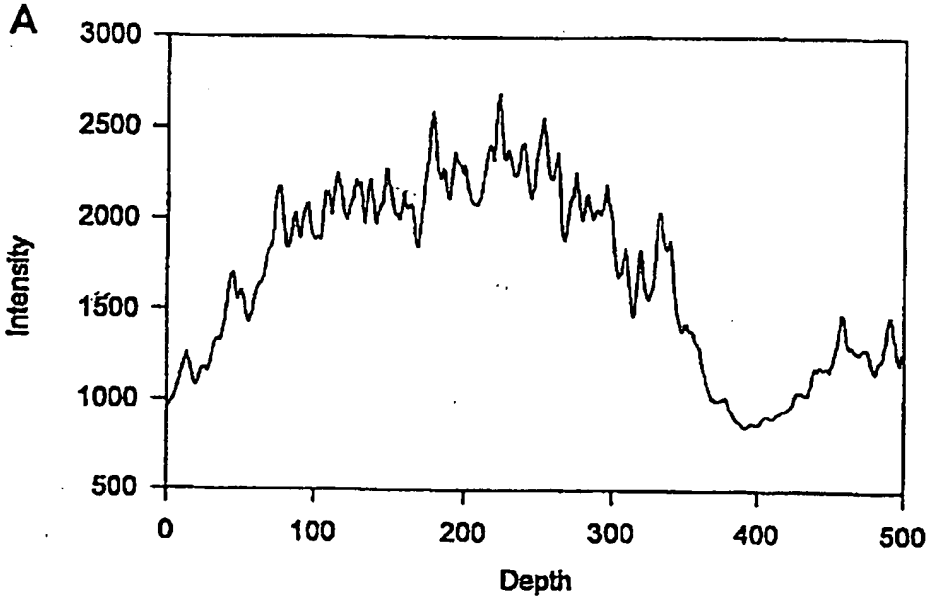
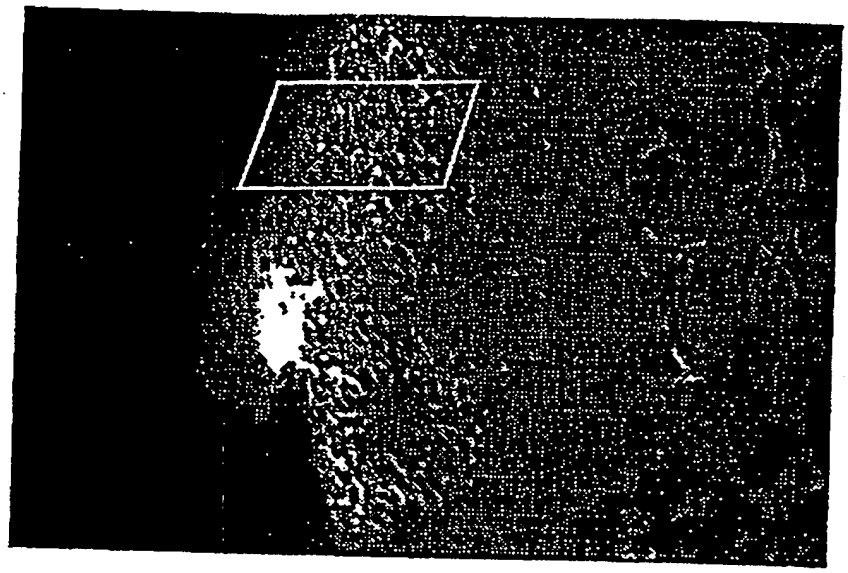


Figure 14

B



158-1

A

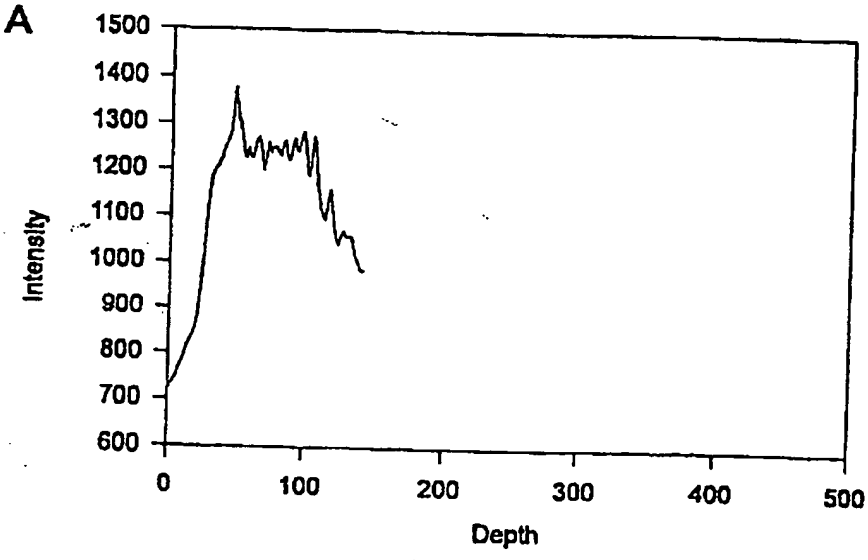
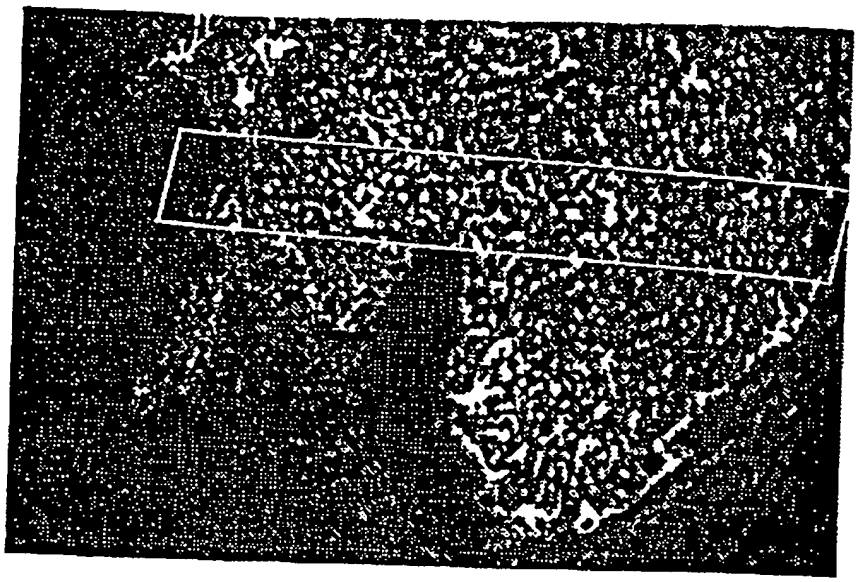


Figure 15

B



159-4

A

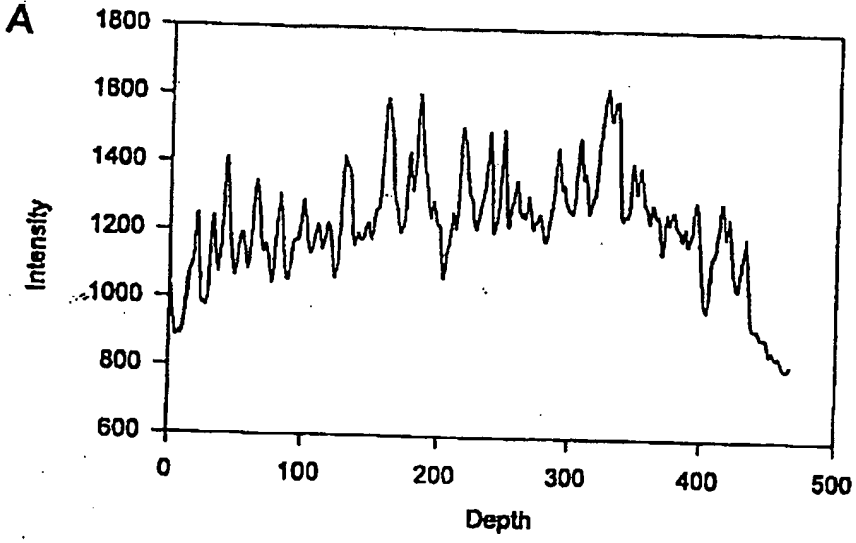
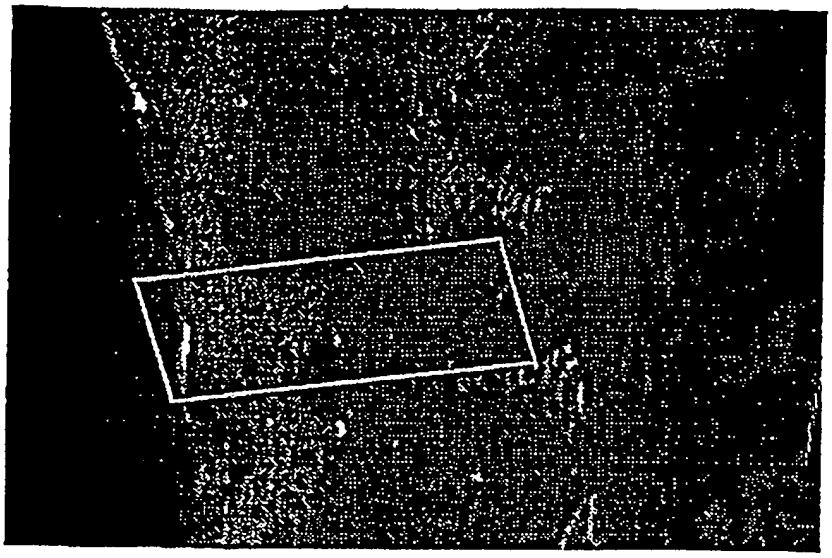


Figure 16

B



A

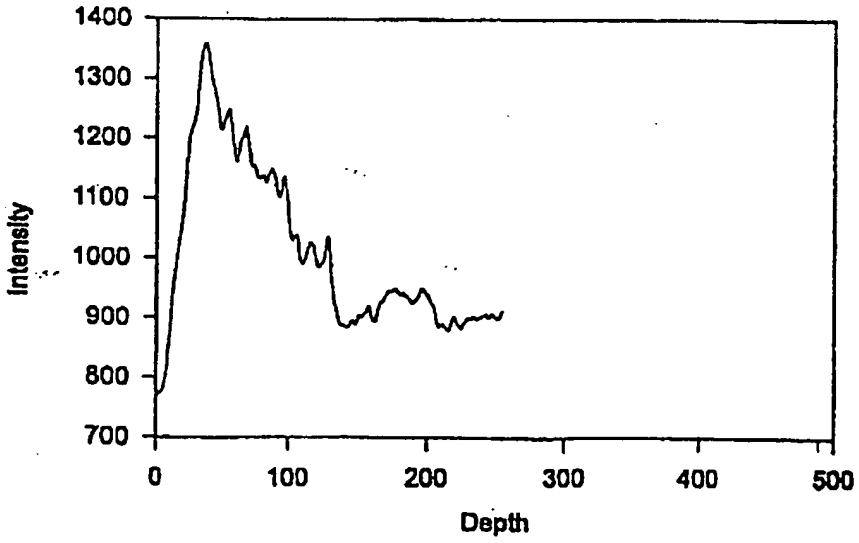
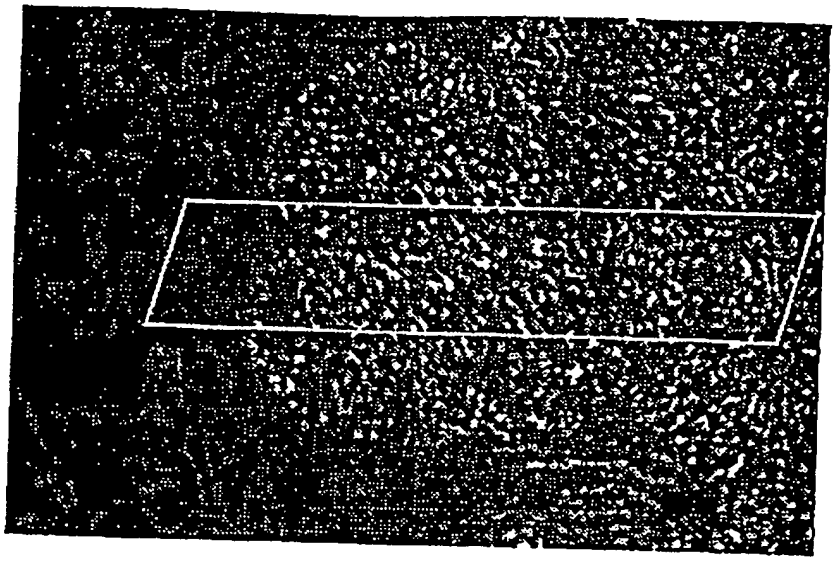

160-1
1400
1300
1200
1100
1000
900
800
700
Intensity
0
100
200
300
400
500
Depth

Figure 17

B



161-6

A

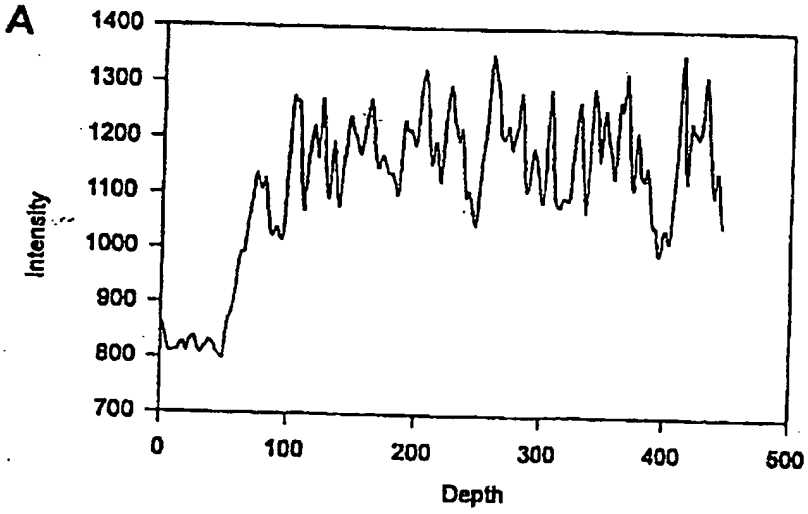
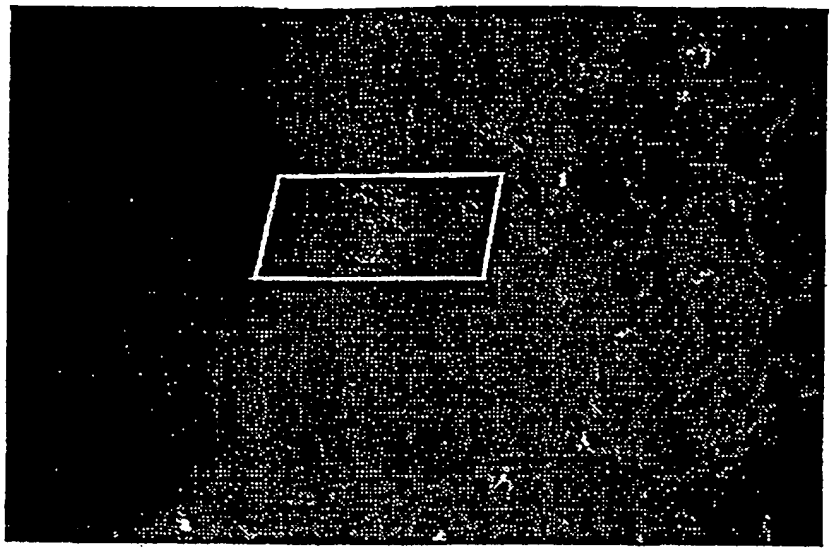


Figure 18

B



162-1

A

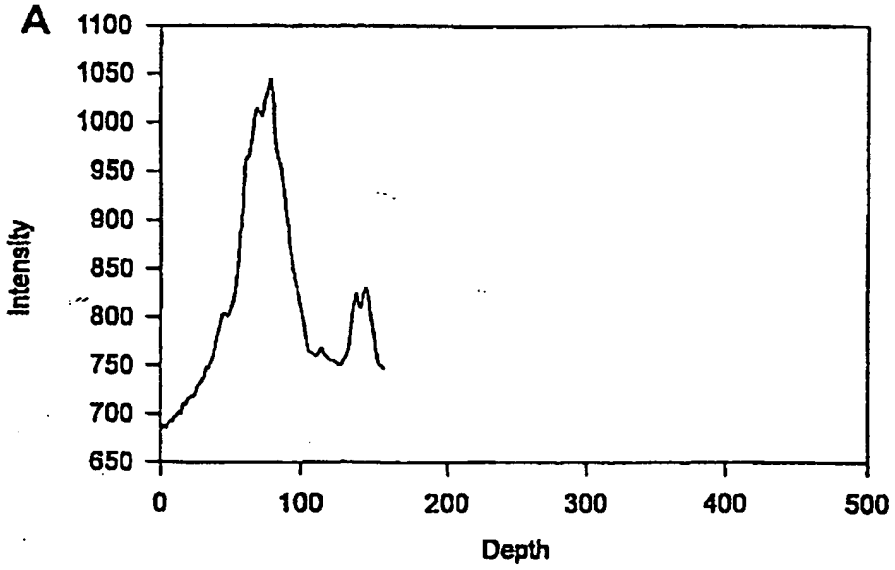


Figure 19

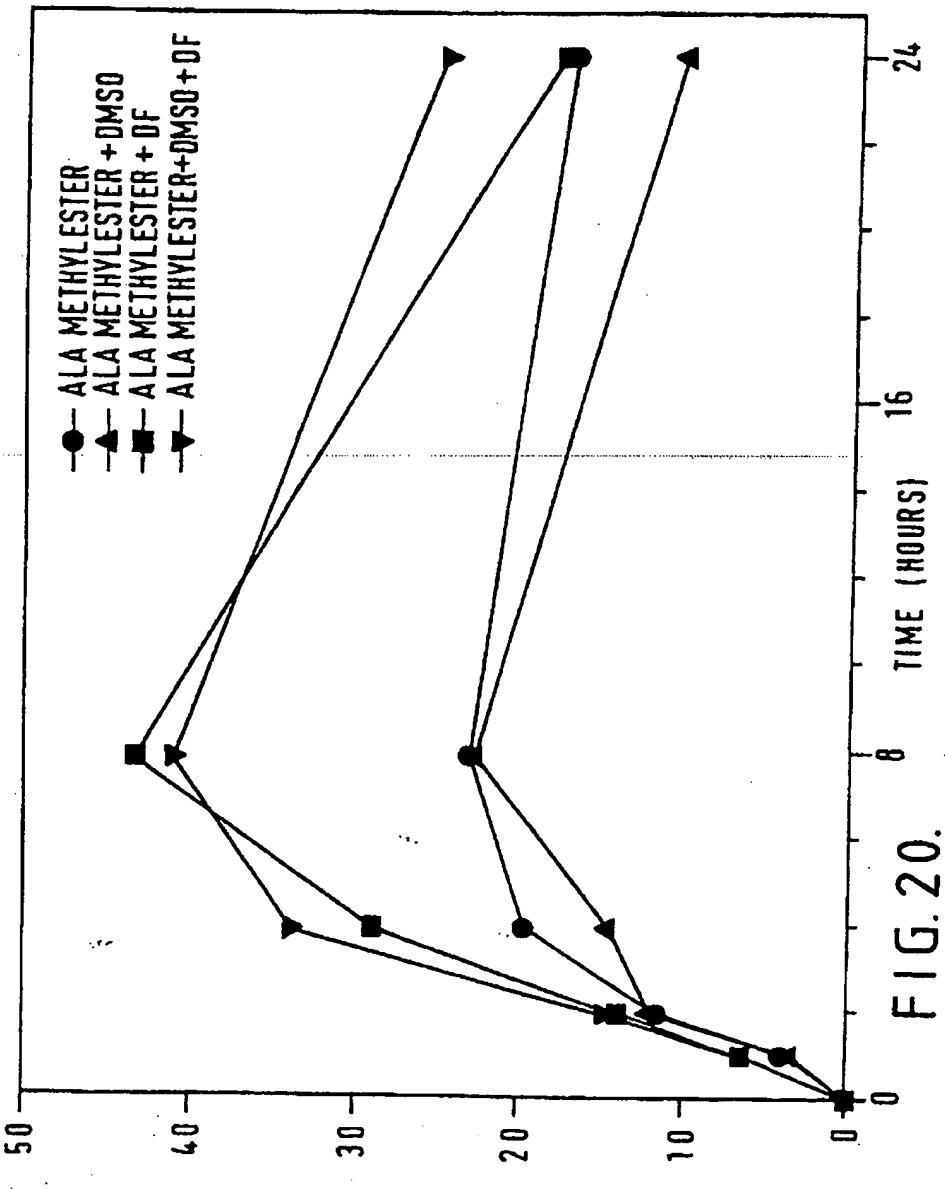

ALA METHYLESTER
ALA METHYLESTER + DMSO
ALA METHYLESTER + DF
ALA METHYLESTER+DMSO+DF
50
40
30
20
10
0
0
8
16
24
TIME (HOURS)

FIG. 20.

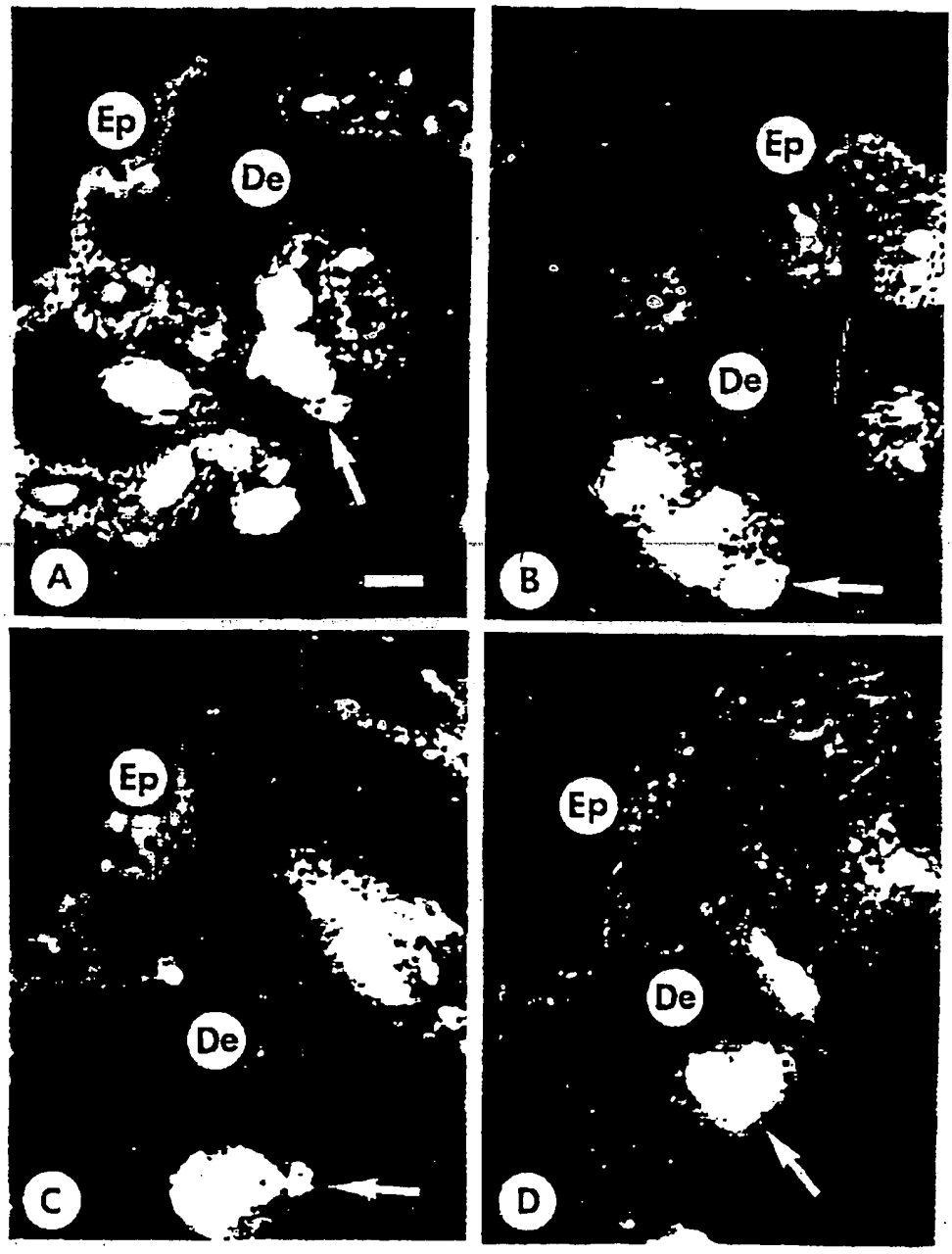


FIG. 21.

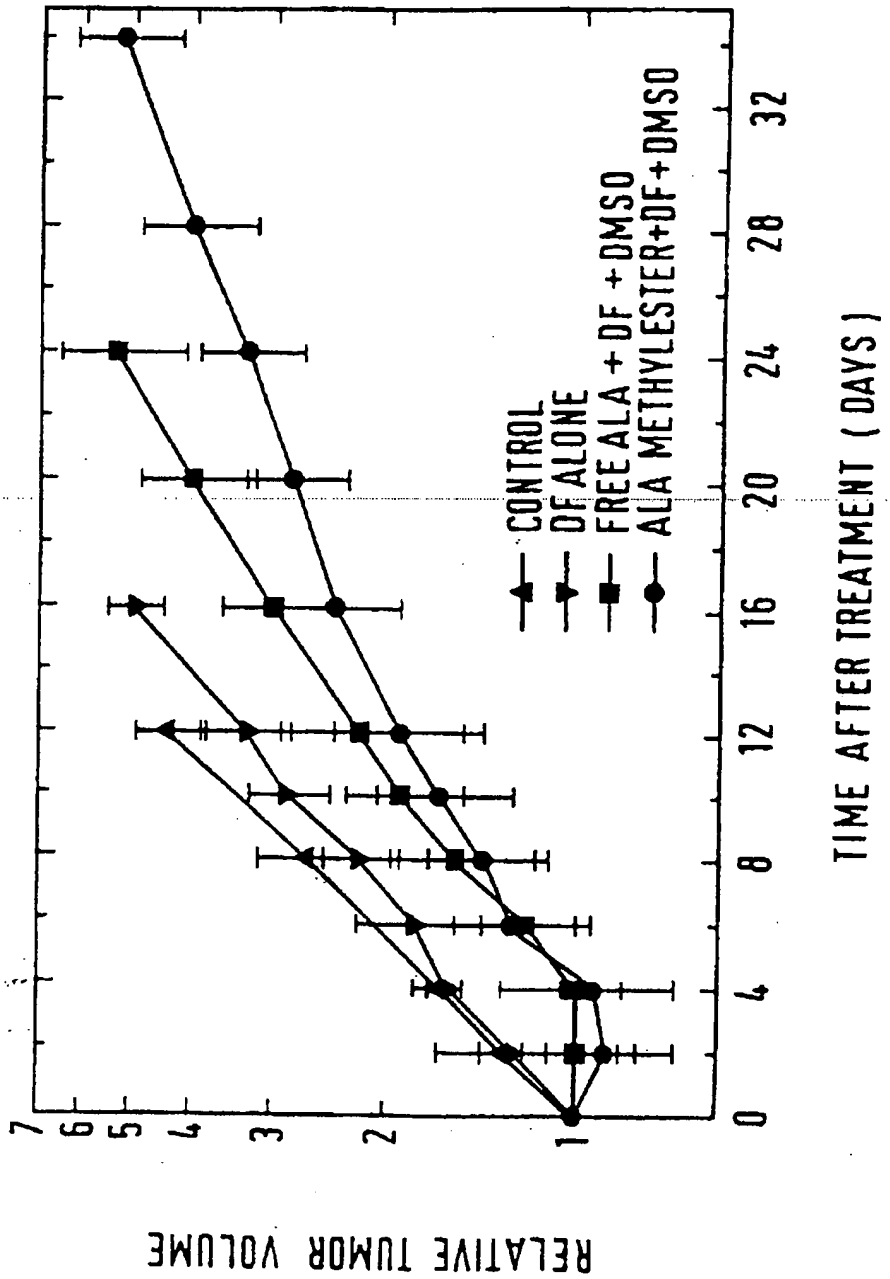

RELATIVE TUMOR VOLUME
7
6
5
4
3
2
1
0
4
8
12
16
20
24
28
32
TIME AFTER TREATMENT (DAYS)
CONTROL
DF ALONE
FREE ALA + DF + DMSO
ALA METHYLESTER+DF+DMSO

FIG. 22.

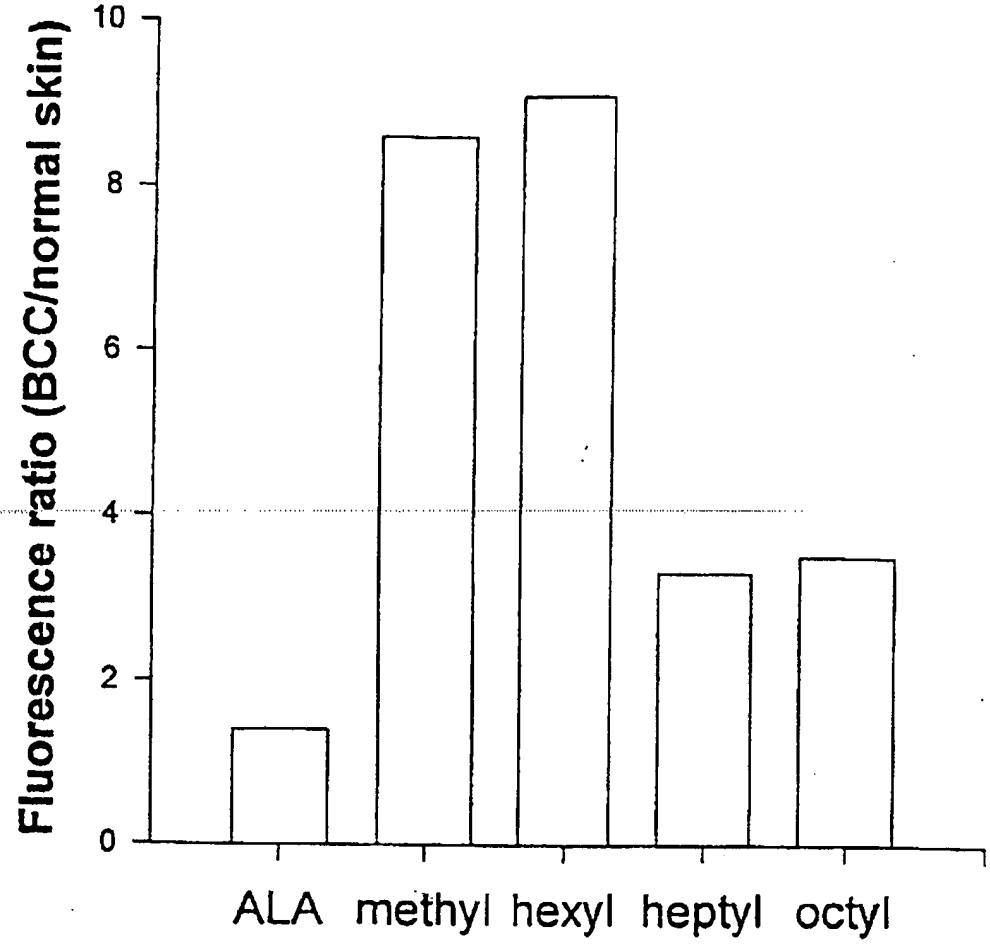

Fluorescence ratio (BCC/normal skin)
10
8
6
4
2
0
ALA
methyl
hexyl
heptyl
octyl

Figure 23

ESTERS OF 5-AMINOLEVULINIC ACID AS PHOTOSENSITIZING AGENTS IN PHOTOCHEMOTHERAPY

The present invention relates to derivatives of 5-aminolevulinic acid (ALA) and in particular to esters of ALA for use as photosensitizing agents in photochemotherapy or diagnosis.

Photochemotherapy, or photodynamic therapy (PDT) as it is also known, is a recently up-coming technique for the treatment of various abnormalities or disorders of the skin or other epithelial organs or mucosa, especially cancers or pre-cancerous lesions, as well as certain non-malignant lesions for example skin complaints such as psoriasis. Photochemotherapy involves the application of photosensitizing (photochemotherapeutic) agents to the affected area of the body, followed by exposure to photoactivating light in order to activate the photosensitizing agents and convert them into cytotoxic form, whereby the affected cells are killed or their proliferative potential diminished.

A range of photosensitizing agents are known, including notably the psoralens, the porphyrins, the chlorins and the phthalocyanins. Such drugs become toxic when exposed to light.

Photosensitizing drugs may exert their effects by a variety of mechanisms, directly or indirectly. Thus for example, certain photosensitizers become directly toxic when activated by light, whereas others act to generate toxic species, e.g. oxidising agents such as singlet oxygen or other oxygen-derived free radicals, which are extremely destructive to cellular material and biomolecules such as lipids, proteins and nucleic acids. Psoralens are an example of directly acting photosensitizers; upon exposure to light they form adducts and cross-links between the two strands of DNA molecules, thereby inhibiting DNA synthesis. The unfortunate risk with this therapy is that unwanted mutagenic and carcinogenic side effects may occur.

This disadvantage may be avoided by selecting photosensitizers with an alternative, indirect mode of action. For example porphyrins, which act indirectly by generation of toxic oxygen species, have no mutagenic side effects and represent more favourable candidates for photochemotherapy. Porphyrins are naturally occurring precursors in the synthesis of heme. In particular, heme is produced when iron (Fe^{3+}) is incorporated in protoporphyrin IX (Pp) by the action of the enzyme ferrochelatase. Pp is an extremely potent photosensitizer, whereas heme has no photosensitizing effect.

One such porphyrin-based drug, Photofrin, has recently been approved as a photosensitizer in the therapy of certain cancers. The main disadvantage is that since it must be administered parenterally, generally intravenously, cause photosensitization of the skin which may last for several weeks following i.v. injection. Photofrin consists of large oligomers of porphyrin and it does not readily penetrate the skin when applied topically. Similar problems exist with other porphyrin-based photosensitizers such as the so-called "hematoporphyrin derivative" (Hpd) which has also been reported for use in cancer photochemotherapy (see for example S. Dougherty. J. Natl. Cancer Ins., 1974, 52; 1333; Kelly and Snell, J. Urol, 1976, 115: 150). Hpd is a complex mixture obtained by treating haematoporphyrin with acetic and sulphuric acids, after which the acetylated product is dissolved with alkali.

To overcome these problems, precursors of Pp have been investigated for photochemotherapeutic potential. In particular the Pp precursor 5-aminolevulinic acid (ALA) has

been investigated as a photochemotherapeutic agent for certain skin cancers. ALA, which is formed from succinyl CoA and glycine in the first step of heme synthesis, is to a limited extent able to penetrate the skin and lead to a localised build-up of Pp; since the action of ferrochelatase (the metallating enzyme) is the rate limiting step in heme synthesis, an excess of ALA leads to accumulation of Pp, the photosensitizing agent. Thus, by applying ALA topically to skin tumours, and then after several hours exposing the tumours to light, a beneficial photochemotherapeutic effect may be obtained (see for example WO91/01727). Since the skin covering basilomas and squamous cell carcinomas is more readily penetrated by ALA than healthy skin, and since the concentration of ferrochelatase is low in skin tumours, it has been found that topical application of ALA leads to a selectively enhanced production of Pp in tumours.

However, whilst the use of ALA represents a significant advance in the art, photochemotherapy with ALA is not always entirely satisfactory. ALA is not able to penetrate all tumours and other tissues with sufficient efficacy to enable treatment of a wide range of tumours or other conditions and ALA also tends to be unstable in pharmaceutical formulations. A need therefore exists for improved photochemotherapeutic agents.

The present invention addresses this need and in particular aims to provide photochemotherapeutic agents which are better able to penetrate the tumour or other abnormality, and which have an enhanced photochemotherapeutic effect over those described in the prior art.

In one aspect, the present invention thus provides compounds being esters of 5-aminolevulinic acids or pharmaceutically acceptable salts thereof for use in photochemotherapy or diagnosis.

In the esters of the invention the 5-amino group may be substituted or unsubstituted, the latter case being the ALA esters.

More particularly, the compounds for use according to the invention are esters of 5-aminolevulinic acids with optionally substituted alkanols, ie. alkyl esters or substituted alkyl esters.

Database Xfire, entries 3060978, 5347132, 5499790, 5620924, 5633390, 5991317 and 6517740 (Beilstein); Cosmo Sogo Kenkyusho KK, Patent Abstracts of Japan, Vol 16; No. 156 (C-0930), 16.4.1992; EP-A-316179 (Tokuyama Soda KK); GB-A-2058077 (Hudson et al) and DE-A-2411382 (Boehringer Sohn Ingelheim) describe alkyl ester derivative of 5-aminolevulinic acid, and derivatives and salts thereof and processes for their preparation.

Alternatively viewed, the invention can therefore be seen to provide compounds of formula I,

$$R_2^2N—CH_2COCH_2—CH_2CO—OR^1 \quad (I)$$

(wherein R^1 may represent alkyl optionally substituted by hydroxy, alkoxy, acyloxy, alkoxycarbonyloxy, amino, aryl, oxo or fluoro groups and optionally interrupted by oxygen, nitrogen, sulphur or phosphorus atoms; and R^2, each of which may be the same or different, represents a hydrogen atom or a group R^1) and salts thereof for use in photochemotherapy or diagnosis.

The substituted alkyl R^1 groups may be mono or polysubstituted. Thus suitable R^1 groups include for example unsubstituted alkyl, alkoxyalkyl, hydroxyalkoxyalkyl, polyhydroxyalkyl, hydroxy poly alkyleneoxyalkyl and the like. The term "acyl" as used herein includes both carboxylate and carbonate groups, thus, acyloxy substituted alkyl groups include for example alkylcarbonyloxy alkyl. In such groups any alkylene moieties preferably have carbon atom

contents defined for alkyl groups below. Preferred aryl groups include phenyl and monocyclic 5–7 membered heteroaromatics, especially phenyl and such groups may themselves optionally be substituted.

Representative substituted alkyl groups R^1 include alkoxymethyl, alkoxyethyl and alkoxypropyl groups or acyloxymethyl, acyloxyethyl and acyloxypropyl groups eg. pivaloyloxymethyl.

Preferred compounds for use according to the invention, include those wherein R^1 represents an unsubstituted alkyl group and/or each R^2 represents a hydrogen atom.

As used herein, the term "alkyl" includes any long or short chain, straight-chained or branched aliphatic saturated or unsaturated hydrocarbon group. The unsaturated alkyl groups may be mono- or polyunsaturated and include both alkenyl and alkynyl groups. Such groups may contain up to 40 carbon atoms. However, alkyl groups containing up to 10 eg. 8, more preferably up to 6, and especially preferably up to 4 carbon atoms are preferred.

Particular mention may be made of ALA-methylester, ALA-ethylester, ALA-propylester, ALA-hexylester, ALA-heptylester and ALA-octylester and salts thereof, which represent preferred compounds for use according to the invention.

The compounds for use in the invention may be prepared using standard processes and procedures well-known in the art for derivatization of multi-functional compounds, and especially esterification. As known in the art, such esterification of compounds may involve protection and deprotection of appropriate groups such that only the required groups remain active and take part in the reaction under the conditions of the esterification. Thus for example the substituents of substituted alkanols used to prepare the esters may be protected during esterification. Similarly the NR_2^2 group on the compound contributing this group to compounds of formula I may be protected during the reaction and deprotected thereafter. Such protection/deprotection procedures are well known in the art for the preparation of derivatives, and in particular, esters of well known amino-acids, see for example Mcomie in "Protective Groups in Organic Chemistry", Plenum, 1973 and T. W. Greene in "Protective Groups in Organic Chemistry", Wiley-Interscience, 1981.

In a further aspect, the present invention thus provides a process for preparing the compounds for use in the invention, comprising forming an ester of the carboxy group of a 5-aminolevulinic acid.

The invention can thus be seen to provide a process for preparing the compounds for use in the invention, comprising reacting a 5-aminolevulinic acid, or an esterifiable derivative thereof, with an alkanol or an ester-forming derivative thereof.

More particularly, this aspect of the invention provides a process for preparing compounds of formula I, which process comprises at least one of the following steps:

(a) reacting a compound of formula II

$$R_2^2N—CH_2COCH_2—CH_2CO—X \quad (II)$$

(wherein X represents a leaving group, for example a hydroxyl group, a halogen atom or alkoxy group or COX represents an acid anhydride group and R^2 is as hereinbefore defined)

with a compound of formula III

$$R^1—OH \quad (III)$$

(wherein R^1 is as hereinbefore defined); and

(b) converting a compound of formula I into a pharmaceutically acceptable salt thereof.

The reaction of step (a) may conveniently be carried out in a solvent or mixture of solvents such as water, acetone, diethylether, methylformamide, tetrahydrofuran etc. at temperatures up to the boiling point of the mixture, preferably at ambient temperatures.

The conditions of the esterification reactions will depend of the alcohol used and the conditions may be chosen such that maximum yield of the ester is obtained. Since the esterification reactions are reversible equilibrium reactions, reaction conditions may be selected in such a way that maximum yield of the ester product is obtained. Such conditions may be obtained by selecting a solvent which is capable of removing the water formed in a typical esterification reaction by forming an azeotrope with water. Such solvents are exemplified by aromatic hydrocarbons or others capable of forming azeotropes with water, e.g. some chlorinated hydrocarbons such as chloroform. For the formation of the lower esters of 5-ALA the equilibrium reaction may be driven to the ester side by using a large excess of the alcohol. With other esters the equilibrium may be driven towards the ester product by using a large excess of the acid.

Esterification reactions are well-known in the art for example, as described by Saul Patai in "The chemistry of the carboxylic acids and esters", (Ch. 11, p. 505, Interscience 1969) and Houban Weyl, (Methoden der Organische Chemie, Band E5, "Carbonsauren und carbonsaurenderivate", p. 504, Georg Thieme Verlag, 1985). The formation of derivatives of amino-acids are described in Band XI/2 of the same series, (Houben Weyl, Methoden der Organische Chemie, Band XI/2, "Stickstoffverbindungen", p. 269, Georg Thieme Verlag, 1958).

The reaction will conveniently be carried out in the presence of a catalyst, eg. an inorganic or organic acid or an acid binding agent such as a base.

The compounds used as starting materials are known from the literature, and in many cases commercially available, or may be obtained using methods known per se. ALA, for example, is available from Sigma or from Photocure, Oslo, Norway.

As mentioned above, the compounds for use according to the invention may take the form of pharmaceutically acceptable salts. Such salts preferably are acid addition salts with physiologically acceptable organic or inorganic acids. Suitable acids include, for example, hydrochloric, hydrobromic, sulphuric, phosphoric, acetic, lactic, citric, tartaric, succinic, maleic, fumaric and ascorbic acids. Procedures for salt formation are conventional in the art.

As mentioned above, the compounds for use according to the invention and their salts have valuable pharmacological properties, namely a photosensitizing agent which renders them useful as photochemotherapeutic agents.

Like ALA, the compounds exert their effects by enhancing production of Pp; upon delivery to the desired site of action hydrolytic enzymes such as esterases present in the target cells break down the esters into the parent ALA, which then enters the haem synthesis pathway and leads to a build-up of Pp. However, the compounds for use according to the invention have a number of advantages over ALA itself. Firstly, the compounds are better able to penetrate skin and other tissues as compared with ALA; the penetration is both deeper and faster. This is an important advantage, especially for topical administration. Secondly, the esters have surprisingly been found to be better enhancers of Pp production than ALA; Pp production levels following administration of the ALA esters are higher than with ALA alone. Thirdly, the compounds for use in the invention demonstrate improved selectivity for the target tissue to be

treated, namely the Pp production-enhancing effect is localised to the desired target lesion and does not spread to the surrounding tissues. This is especially evident with tumours. Finally, the compounds appear to localise better to the target tissue upon administration. This is especially important for systemic application, since it means that undesirable photosensitization effects, as reported in the literature for other porphyrin-based photosensitizers, may be reduced or avoided.

A further aspect of the present invention accordingly provides a pharmaceutical composition comprising a compound as described hereinbefore, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

In a still further aspect, there is also provided the use of a compound as described hereinbefore, or a pharmaceutically acceptable salt thereof, for the preparation of a therapeutic agent for use in photochemotherapy, and especially for the treatment of disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

The abnormalities and disorders which may be treated according to the present invention include any malignant, pre-malignant and non-malignant abnormalities or disorders responsive to photochemotherapy eg. tumours or other growths, skin disorders such as psoriasis or actinic keratoses, skin abrasions, and other diseases or infections eg. bacterial, viral or fungal infections, for example Herpes virus infections. The invention is particularly suited to the treatment of diseases, disorders or abnormalities where discrete lesions are formed to which the compositions may be directly applied (lesions is used here in a broad sense to include tumours and the like).

The internal and external body surfaces which may be treated according to the invention include the skin and all other epithelial and serosal surfaces, including for example mucosa, the linings of organs eg. the respiratory, gastrointestinal and genito-urinary tracts, and glands with ducts which empty onto such surfaces (e.g. liver, hair follicles with sebaceous glands, mammary glands, salivary glands and seminal vesicles). In addition to the skin, such surfaces include for example the lining of the vagina, the endometrium and the urothelium. Such surfaces may also include cavities formed in the body following excision of diseased or cancerous tissue eg. brain cavities following the excision of tumours such as gliomas.

Exemplary surfaces thus include: (i) skin and conjunctiva; (ii) the lining of the mouth, pharynx, oesophagus, stomach, intestines and intestinal appendages, rectum, and anal canal; (iii) the lining of the nasal passages, nasal sinuses, nasopharynx, trachea, bronchi, and bronchioles; (iv) the lining of the ureters, urinary bladder, and urethra; (v) the lining of the vagina, uterine cervix, and uterus; (vi) the parietal and visceral pleura; (vii) the lining of the peritoneal and pelvic cavities, and the surface of the organs contained within those cavities; (viii) the dura mater and meninges; (ix) any tumors in solid tissues that can be made accessible to photoactivating light e.g. either directly, at time of surgery, or via an optical fibre inserted through a needle.

The compositions of the invention may be formulated in conventional manner with one or more physiologically acceptable carriers or excipients, according to techniques well known in the art. Compositions may be administered topically, orally or systemically. Topical compositions are preferred, and include gels, creams, ointments, sprays, lotions, salves, sticks, soaps, powders, pessaries, aerosols, drops and any of the other conventional pharmaceutical forms in the art.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will, in general, also contain one or more emulsifying, dispersing, suspending, thickening or colouring agents. Powders may be formed with the aid of any suitable powder base. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing, solubilising or suspending agents. Aerosol sprays are conveniently delivered from pressurised packs, with the use of a suitable propellant.

Alternatively, the compositions may be provided in a form adapted for oral or parenteral administration, for example by intradermal, subcutaneous, intraperitoneal or intravenous injection. Alternative pharmaceutical forms thus include plain or coated tablets, capsules, suspensions and solutions containing the active component optionally together with one or more inert conventional carriers and/or diluents, e.g. with corn starch, lactose, sucrose, microcrystalline cellulose, magnesium stearate, polyvinylpyrrolidone, citric acid, tartaric acid, water, water/ethanol, water/glycerol, water/sorbitol, water/polyethyleneglycol, propyleneglycol, stearylalcohol, carboxymethylcellulose or fatty substances such as hard fat or suitable mixtures thereof.

The concentration of the compounds as described hereinbefore in the compositions, depends upon the nature of the compound, the composition, mode of administration and the patient and may be varied or adjusted according to choice. Generally however, concentration ranges of 1 to 50% (w/w) are suitable. For therapeutic applications concentration ranges of 10 to 50% have been found to be suitable, eg. 15 to 30% (w/w).

Following administration to the surface, the area treated is exposed to light to achieve the photochemotherapeutic effect. The length of time following administration, at which the light exposure takes place will depend on the nature of the composition and the form of administration. This can generally be in the order of 0.5 to 48 hours, e.g. 1 to 10 hours.

The irradiation will in general be applied at a dose level of 40 to 200 Joules/cm^2, for example at 100 Joules/cm^2.

The wavelength of light used for irradiation may be selected to achieve a more efficacious photochemotherapeutic effect. Conventionally, when porphyrins are used in photochemotherapy they are irradiated with light at about the absorption maximum of the porphyrin. Thus, for example in the case of the prior art use of ALA in photochemotherapy of skin cancer, wavelengths in the region 350–640 nm, preferably 610–635 nm were employed. However, by selecting a broad range of wavelengths for irradiation, extending beyond the absorption maximum of the porphyrin, the photosensitizing effect may be enhanced. Whilst not wishing to be bound by theory, this is thought to be due to the fact that when Pp, and other porphyrins, are exposed to light having wavelengths within its absorption spectrum, it is degraded into various photo-products including in particular photoprotoporphyrin (PPp). PPp is a chlorin and has a considerable photo-sensitizing effect; its absorption spectrum stretches out to longer wavelengths beyond the wavelengths at which Pp absorbs ie. up to almost 700 nm (Pp absorbs almost no light above 650 nm). Thus in conventional photochemotherapy, the wavelengths used do not excite PPp and hence do not obtain the benefit of its additional photosensitizing effect. Irradiation with wavelengths of light in the range 500–700 nm has been found to be particularly effective. It is particularly important to include the wavelengths 630 and 690 nm.

A further aspect of the invention thus provides a method of photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body, comprising administering to the affected surfaces, a composition as hereinbefore defined, and exposing said surfaces to light, preferably to light in the wavelength region 300–800 nm, for example 500–700 nm.

Methods for irradiation of different areas of the body, eg. by lamps or lasers are well known in the art (see for example Van den Bergh, Chemistry in Britain, May 1986 p. 430–439).

The compounds for use in the invention may be formulated and/or administered with other photosensitizing agents, for example ALA or photofrin, or with other active components which may enhance the photochemotherapeutic effect. For example, chelating agents may beneficially be included in order to enhance accumulation of Pp; the chelation of iron by the chelating agents prevents its incorporation into Pp to form haem by the action of the enzyme ferrochelatase, thereby leading to a build-up of Pp. The photosensitizing effect is thus enhanced.

Aminopolycarboxylic acid chelating agents are particularly suitable for use in this regard, including any of the chelants described in the literature for metal detoxification or for the chelation of paramagnetic metal ions in magnetic resonance imaging contrast agents. Particular mention may be made of EDTA, CDTA (cyclohexane diamine tetraacetic acid), DTPA and DOTA. EDTA is preferred. To achieve the iron-chelating effect, desferrioxamine and other siderophores may also be used, e.g. in conjunction with aminopolycarboxylic acid chelating agents such as EDTA.

The chelating agent may conveniently be used at a concentration of 1 to 20% eg. 2 to 10% (w/w).

Additionally, it has been found that surface-penetration assisting agents and especially dialkylsuphoxides such as dimethylsulphoxide (DMSO) may have a beneficial effect in enhancing the photochemotherapeutic effect. This is described in detail in our co-pending application No. PCT/GB94/01951, a copy of the specification of which is appended hereto.

The surface-penetration assisting agent may be any of the skin-penetration assisting agents described in the pharmaceutical literature e.g. HPE-101 (available from Hisamitsu), DMSO and other dialkylsulphoxides, in particular n-decylmethyl-sulphoxide (NDMS), dimethylsulphacetamide, dimethylformamide (DMFA), dimethylacetamide, glycols, various pyrrolidone derivatives (Woodford et al., J. Toxicol. Cut. & Ocular Toxicology, 1986, 5: 167–177), and Azone® (Stoughton et al., Drug Dpv. Ind. Pharm. 1983, 9: 725–744), or mixtures thereof.

DMSO however has a number of beneficial effects and is strongly preferred. Thus, in addition to the surface-penetration assisting effect (DMSO is particularly effective in enhancing the depth of penetration of the active agent into the tissue), DMSO has anti-histamine and anti-inflammatory activities. In addition, DMSO has been found to increase the activity of the enzymes ALA-synthase and ALA-dehydrogenase (the enzymes which, respectively, form and condense ALA to porphobilinogen) thereby enhancing the formation of the active form, Pp.

The surface penetration agent may conveniently be provided in a concentration range of 2 to 50% (w/w), eg about 10% (w/w).

According to the condition being treated, and the nature of the composition, the compounds for use in the invention may be co-administered with such other optional agents, for example in a single composition or they may be administered sequentially or separately. Indeed, in many cases a particularly beneficial photochemotherapeutic effect may be obtained by pre-treatment with the surface-penetration assisting agent in a separate step, prior to administration of the compounds for use in the invention. Furthermore, in some situations a pre-treatment with the surface-penetration assisting agent, followed by administration of the photochemotherapeutic agent in conjunction with the surface-penetration assisting agent may be beneficial. When a surface-penetration assisting agent is used in pre-treatment this may be used at high concentrations, e.g. up to 100% (w/w). If such a pre-treatment step is employed, the photochemotherapeutic agent may subsequently be administered up to several hours following pre-treatment eg. at an interval of 5–60 minutes following pre-treatment.

Viewed from a further aspect, the invention thus provides a product comprising a compound as described hereinbefore or a pharmaceutically acceptable salt thereof, together with at least one surface-penetration assisting agent, and optionally one or more chelating agents as a combined preparation for simultaneous, separate or sequential use in treating disorders or abnormalities of external or internal surfaces of the body which are responsive to photochemotherapy.

Alternatively viewed, this aspect of the invention also provides a kit for use in photochemotherapy of disorders or abnormalities of external or internal surfaces of the body comprising:

a) a first container containing a compound as described hereinbefore or a pharmaceutically acceptable salt thereof,

b) a second container containing at least one surface penetration assisting agent; and optionally

c) one or more chelating agents contained either within said first container or in a third container.

Where the surface penetration agent is applied in a separate pre-treatment step, it may be applied in higher concentration, for example up to 100% (w/w).

It will be appreciated that the method of therapy using compounds as described hereinbefore inevitably involves the fluorescence of the disorder or abnormality to be treated. Whilst the intensity of this fluorescence may be used to eliminate abnormal cells, the localization of the fluorescence may be used to visualize the size, extent and situation of the abnormality or disorder. This is made possible through the surprising ability of ALA esters to preferentially localize to non-normal tissue.

The abnormality or disorder thus identified or confirmed at the site of investigation may then be treated through alternative therapeutic techniques e.g. surgical or chemical treatment, or by the method of therapy of the invention by continued build up of fluorescence or through further application of compounds of the invention at the appropriate site. It will be appreciated that diagnostic techniques may require lower levels of fluorescence for visualization than used in therapeutic treatments. Thus, generally, concentration ranges of 1 to 50% e.g. 1–5% (w/w) are suitable. Sites, methods and modes of administration have been considered before with regard to the therapeutic uses and are applicable also to diagnostic uses described here. The compounds for use in the invention may also be used for in vitro diagnostic techniques, for example for examination of the cells contained in body fluids. The higher fluoresence associated with non-normal tissue may conveniently be indicative of an abnormality or disorder. This method is highly sensitive and may be used for early detection of abnormalities or disorders, for example bladder or lung carcinoma by examination of the epithelial cells in urine or sputum samples,

respectively. Other useful body fluids which may be used for diagnosis in addition to urine and sputum include blood, semen, tears, spinal fluid etc. Tissue samples or preparations may also be evaluated, for example biopsy tissue or bone marrow samples. The present invention thus extends to the use of compounds of the invention, or salts thereof for diagnosis according to the aforementioned methods for photochemotherapy, and products and kits for performing said diagnosis.

A further aspect of the invention relates to a method of in vitro diagnosis, of abnormalities or disorders by assaying a sample of body fluid or tissue of a patient, said method comprising at least the following steps:

i) admixing said body fluid or tissue with a compound as described hereinbefore,

ii) exposing said mixture to light,

iii) ascertaining the level of fluorescence, and

iv) comparing the level of fluorescence to control levels.

The invention will now be described in more detail in the following non-limiting Examples, with reference to the drawings in which:

FIG. 1 shows fluorescence intensity (relative units vs wavelength (nm)) of PpIX in the normal skin of mice after topical administration of

(A) free ALA

(B) ALA methylester

(C) ALA ethylester

(D) ALA propylester

after 0.5, 1, 1.5, 2.5, 3, 3.5 and 14 hours following administration;

FIG. 2 shows the distribution of PpIX as measured by fluorescence intensity (relative units vs wavelength (nm)) in Brain, dermis, Ear, Liver and muscle 14 hours after topical administration to the normal skin of mice:

(A) free ALA

(B) ALA methylester

(C) ALA ethylester

(D) ALA propylester;

FIG. 3 shows PpIX fluorescence (fluorescence intesity, relative units vs wavelength (nm)) in the skin of mice 15 minutes, 1 hour, 4 hours and 10 hours after intraperitoneal injection of ALA methylester (150 mg/kg);

FIG. 4 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA methylester to basal cell carcinoma (BCC) lesions on the skin of human patients (- tumour; — normal skin);

FIG. 5 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA ethylester to basal cell carcinoma (BCC) lesions on the skin of human patients (- tumour; — normal skin);

FIG. 6 shows PPIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA propylester to basal cell carcinoma (BCC) lesions on the skin of human patients (- tumour; — normal skin);

FIG. 7 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) (A) 1.5 hours and (B) 4 hours after topical administration of ALA to basal cell carinoma (BCC) lesions on the skin of human patients (- tumour; — normal skin);

FIG. 8 shows measurement of PpIX production following topical application of ALA methylester in human BCC and surrounding normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 9 shows measurement of PpIX production following topical application of ALA in human BCC and surrounding normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 10 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) 24 hours following topical administration of ALA methylester to BCC lesion and to normal skin of human patients.

FIG. 11 shows PpIX fluorescence (fluorescence intensity relative units vs wavelength (nm)) 24 hours following topical administration of ALA to BCC lesion and to normal skin of human patients.

FIG. 12 shows measurement of PpIX production 4.5 hours following topical application of ALA methylester in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 13 shows measurement of PpIX production 4.5 hours following topical application of ALA methylester in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 14 shows measurement of PpIX production 24 hours following topical application of ALA methylester in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 15 shows measurement of PpIX production 24 hours following topical application of ALA methylester in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 16 shows measurement of PpIX production 24 hours following topical application of free ALA in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 17 shows measurement of PpIX production 24 hours following topical application of free ALA in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 18 shows measurement of PpIX production 4.5 hours following topical application of free ALA and 20% DMSO in human BCC by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 19 shows measurement of PpIX production 4.5 hours following topical application of free ALA and 20% DMSO in human normal skin by CDD microscopy of biopsies (A) graphical representation showing fluorescence intensity vs depth (μm) and (B) micrograph;

FIG. 20 shows a time course (fluorescence intensity relative units vs time (hours)) of ALA methylester-induced (PpIX) fluorescence in the mouse skin after topical application of ALA methylester alone (-●-), ALA methylester plus DMSO (-▲-), ALA methylester plus desferrioxamine (DF) (-■-) or ALA methylester plus DF and DMSO (-▼-). Each point is the mean of measurements from at least three mice;

FIG. 21 shows fluorescence photographs of the mouse skin taken 1 h after topical application of free ALA alone (A), ALA methylester (B), ALA ethylester (C) and ALA propylester (D), showing fluorescence in the epidermis (Ep),

epithelial hair follicles and sebaceous gland (arrows), but not in the dermis (De). Original magnification×250.

FIG. 22 is a graph showing relative tumour volume against time (days) following treatment of WiDr human colonic carcinoma transplanted subcutaneously into nude mice with ALA or ALA methylester plus DF; (-▲-) control; (-▼-) DF alone; (-■-) ALA+DF+DMSO; (-●-) ALA methylester+DF+DMSO.

FIG. 23 shows ppIX fluoresence ratios between BCC lesions and surrounding normal skin after topical application of ALA or its esters.

EXAMPLE 1

Preparation of Methyl 5-aminolevulinate Hydrochloride

To a 500 ml glass reactor containing 200 ml methanol, was added 1 g 5-amino-levulinic acid hydrochloride and 1 drop conc. HCl. The reaction mixture was then stirred overnight at 60° C. The progress of the esterification was followed by ^{1}H-NMR. Excess methanol was removed by distillation, and the product further dried under vacuum at 30–40° C., giving methyl 5-aminolevulinate hydrochloride. The structure was confirmed by ^{1}H-NMR in DMSO-d_6.

EXAMPLE 2

Preparation of Ethyl 5-aminolevulinate Hydrochloride (ALA ethylester)

1 g 5-aminolevulinic acid hydrochloride was added to 200 ml dry ethanol containing 1–2 drops conc. hydrochloric acid in a 250 ml glass reactor equipped with a stirrer, reflux condenser and a thermometer. The esterification was performed at reflux overnight (70–80° C.). After the reaction had gone to completion, the ethanol was removed under vacuum. Finally, the product was dried under high vacuum at 30–40° C., giving 0.94 g Ethyl 5-aminolevulinate hydrochloride. Confirmation of the structure was done by ^{1}H-NMR in DMSO-d_6.

EXAMPLE 3

Preparation of n-propyl 5-aminolevulinate Hydrochloride (ALA propylester)

0.5 g 5-aminolevulinic acid hydrochloride was dissolved in 100 ml dry n-propanol containing 1–2 drops of conc. hydrochloride in a 250 ml glass reactor equipped with a stirrer, reflux condenser and a thermometer. The reaction mixture was stirred at 70–80° C. for approx. 20 hours. After all the 5-aminolevulinic acid was converted to its n-propylester (followed by ^{1}H-NMR), the excess propanol was removed, and the product dried under high vacuum (<1 mBar) at 40–50° C. The reaction gave 0.49 g propyl 5-aminolevulinate hydrochloride. The structure was confirmed by ^{1}H-NMR in DMSO-d_6.

EXAMPLE 4

Preparation of n-hexyl 5-aminolevulinic Hydrochloride (ALA hexylester)

2 grams of 5-aminolevulinic acid hydrochloride was dissolved in 25 grams of dry n-hexanol with 5–6 drops of conc. hydrochloride added in a 50 ml glass reactor equipped with a reflux condenser and a thermometer. The reaction mixture was held at 50–60° C. for approx. 3 days. The excess n-hexanol was removed under vacuum and the product finally dried under high vacuum, giving 2.4 grams of n-hexyl 5-aminolevulinate hydrochloride. The structure was confirmed by ^{1}H-NMR spectroscopy in DMSO-d_6.

EXAMPLE 5

Preparation of n-heptyl 5-aminolevulinic Hydrochloride (ALA heptylester)

0.5 g 5-aminolevulinic acid hydrochloride was added to 30 grams of n-heptanol containing 5 drops of conc. hydrochloride in a 100 ml glass reactor equipped with a stirrer, reflux condenser and a thermometer. After all the 5-aminolevulinic acid had dissolved, the reaction mixture was stirred at 70–80° C. for approx. 48 hours. After the 5-aminolevulinic acid was converted to its n-heptyl ester (followed by ^{1}H-NMR), the excess alcohol was removed, and the product dried under high vacuum (<1 mbar) at 70° C. The reaction gave 1.5 g n-heptyl 5-aminolevulinate hydrochloride. The structure was confirmed by ^{1}H-NMR in DMSO-d_6.

EXAMPLE 6

Preparation of n-octyl 5-aminolevulinic Hydrochloride (ALA octylester)

1 gram 5-aminolevulinic acid hydrochloride was added to 30 grams of dry n-octanol containing 5–6 drops of conc. hydrochloride in a 50 ml glass reactor equipped with a reflux condenser, stirrer and a thermometer. The reaction mixture was stirred at 65–70° C. for approx. 2 days. Excess n-octanol was removed under vacuum and the product finally dried under high vacuum, giving 1.5 grams of n-octyl 5-aminolevulinate hydrochloride. The structure was confirmed by ^{1}H-NMR spectroscopy in DMSO-d_6.

EXAMPLE 7

Formulation

20% creams were prepared by admixture of the active component, ALA, ALA methylester, ALA ethylester, or ALA propylester (prepared according to Examples 1 to 3 respectively), with "Urguentum Merck" cream base (available from Merck) consisting of silicon dioxide, paraffin liq., vaseline, album, cetostearol., polysorbat. 40, glycerol monostearate, Miglyol®812 (a mixture of plant fatty acids), polypropyleneglycol., and purified water.

Corresponding creams were also prepared, additionally containing 3–20% DMSO.

EXAMPLE 8

Determination of Protoporphyrin IX Production in the Skin of Mice by CCD Microscopy of Biopsies

A commercial oil-in-water cream containing (20% w/w) one of the chemicals (free ALA, ALA methylester, ALA ethylester and ALA propylester) (see Example 1) was topically applied to the normal skin of nu/nu nude mice for 0.5, 1, 3 and 6 hours, then biopsied and evaluated by means of microscopic fluorescence photometry incorporating a light-sensitive thermol-electrically cooled charge coupled device (CCD) camera. The results show that free ALA and its three ester derivatives are taken up by the skin tissue, the esterified ALA derivatives are being deesterified in the skin, and converted into protoporphyrin IX (PpIX) 0.5 hours after topical application. The fluorescence intensity of PpIX in the skin increased with the time of the application and the

maximum amounts of the fluorescence were seen about 6 hours (the latest time point studied) after the application in all cases.

EXAMPLE 9

Measurements in Situ of Protoporphyrin IX Production in the Skin of Mice by an Optical-fiber Based System

The aim of this study was to investigate the build-up of esterified ALA ester-induced porphyrins fluorescence in the normal skin of nude mice in vivo after topical or systemic administration of ALA ester derivatives.

MATERIALS AND METHODS

Chemicals. 5-aminolevulinic acid (ALA) methyl-, ethyl- and propyl-esters ($H_2N—CH_2COOCH_2—CH_2COO—R$; R can be CH_3, $CH_2—CH_2—CH_3$) were prepared by Norsk Hydro Research Center (Porsgrunn, Norway) as described in Examples 1 to 3. Free ALA hydrochloride and desferrioxamine mesylate (DF) were purchased from Sigma Chemical Company (St. Louis, Mo., USA). Dimethyl sulphoxide (DMSO) was obtained from Janssen Chimica (Geel, Belgium). Commercial oil-water creams (Unguentum Merck, Darmstadt, Germany) containing 20% one of the ALA ester derivatives (w/w), 20% free ALA, 20% ALA methylester plus 5% DF, 20% ALA methylester plus 20% DMSO, or 20% ALA methylester plus 5% DF and 20% DMSO were freshly prepared prior to use. All creams were made by the Pharmacy at the Norwegian radium Hospital. For intraperitoneal injection, ALA and its methylester were freshly dissolved in saline. All other chemicals used were of the highest purity commercially available.

Animals. Female Balb/c nu/nu athymic nude mice were obtained from the Animal Laboratory at the Norwegian Radium Hospital and kept under specific-pathogen-free conditions. At the start of the experiments the mice were 6–7 weeks old weighing 18–24 g. Three mice were housed per cage with autoclaved covers in a dark room during the experiments.

Treatment procedure. One of the creams was painted on the normal skin at right flank region of each mouse, and covered by a semi-permeable dressing (3M, St Paul, Minn., USA) for various time intervals (from 0.25 to 24 h) before fluorescence measurements in situ or being biopsied for microscopic fluorescence imaging. About 0.2 g cream was applied to an approximate 2.25 cm^2 area of the skin. In the case of i.p. injection the mice were given ALA or its methylester at a dose of 150 mg/kg. At least three mice were used for each condition.

Fluorescence spectroscopic measurements in situ. A perkin Elmer LS-50 fluorescence spectrometer equipped with a red-sensitive photomultiplier (Hamamatsu R 928) was used. This instrument has a pulsed Xenon arc light source and phase sensitive detection, such that fluorescence can be readily measured. Part of the excitation beam (set at 408 nm for fluorescence measurements) was reflected into a 600 μm core multimodns optical quartz fiber (No. 3501 393, Dornier Medizintechnik, GmbH, Germering, Germany) by means of a mirror for application onto the subject through a hand held probe. Emission in the region of 550–750 nm was measured via emission fibres collecting information through the probe.

Fluorescence microscopy. After the creams were topically applied to the skin of mice for various times (as indicated above), the skin was biopsied and the frozen tissue sections were cut with a cryostat to a thickness of 8 μm. The fluorescence microscopy was carried out using an Axioplan microscope (Zeiss, Germany) with a 100 W mercury lamp. The fluorescence images were recorded by a light-sensitive thermo-electrically cooled charge coupled device (CCD) camera (resolution: 385×578 pixels with a dynamic range of 16 bits per pixel)(Astromed CCD 3200, Cambridge, UK) and hard copies on a video printer (Sony multiscan video printer UP-930). The filter combination used for detection of porphyrin fluorescence consisted of 390–440 nm excitation filter, a 460 nm beam splitter and a >600 nm emission filter.

Results

PPIX fluorescence was measured in situ by an optical-fiber based system in the normal skin of nude mice 0.5, 1, 1.5, 2.5, 3, 3.5 and 14 hours after topical application of free ALA or one of its ester derivatives as described above. As shown in FIG. 1, the PpIX fluorescence was already built-up 1 hour after topical application in the case of all derivatives, while the fluorescence was seen 1.5 hours after the application of free ALA. The maximum fluorescence intensity was found 14 hours after the application in all cases, but PpIX fluorescence induced from ALA esters in the skin was stronger than that from free ALA. Fur thermore, as can be seen in FIG. 2, 14 hours after the application no fluorescence of ALA-esters-induced PpIX was detected in other areas of the skin and internal organs including ear, dermis, muscle, brain and liver. However, in the case of free ALA, a strong fluorescence was also seen in the ear as well as in the other areas of the skin. Thus, after topical application ALA-ester-induced PpIX was found locally in the skin, whereas free ALA-induced PpIX distributed not only locally, but also in other areas of the skin. We suggest that ALA is transported in the blood and that PpIX is subsequently formed in all organs containing the enzymes of the heme synthesis pathway and/or PPIX is formed in the skin and then transported to other tissues via blood circulation. The latter situation may lead to skin photosensitivity in areas where free ALA is not topically applied. In addition, after intraperitoneal injection of ALA methylester at a dose of 150 mg/kg, the PpIX fluorescence in the skin of mice was built-up 15 minutes after the injection and the peak value was found around 4 hours, and the fluorescence disappeared within 10 hours post the injection (FIG. 3). This kinetic pattern is similar to that of the fluorescence of free ALA-induced porphyrins in the skin following i.p. injection of the same dose, although the fluorescence decreased faster in the case of the ester than in the case of the free ALA.

EXAMPLE 10

Measurements of Protoporphyrin IX Production in Human Basal Cell Carcinoma (BCC) and Surrounding Normal Skin by Optical-fiber Based System

The PpIX fluorescence in the BCC lesions and surrounding normal skin of human patients was measured in situ by optical-fiber based system after topical application of 20% free ALA and its derivatives for various time intervals.

FIGS. 4, 5, 6 and 7 show that, compared to free ALA, the ALA derivatives-induced PpIX was built up faster, produced more and localized more selectively in the BCC lesions (i.e. much less fluorescence in the surrounding normal skin), particularly for ALA methylester.

EXAMPLE 11

In Vivo Fluorescence Surface Measurements of PpIX Production in Human BCC and Surrounding Normal Skin by CCD Microscopy of Biopsies

In a 78 years old Caucasian male presenting multiple ulcero-nodular BCCs lesions were exposed to commercial

oil-in-water creams containing either ALA alone (20% w/w) or ALA methyl ester (20% w/w) (as described in Example 7) covered by a semi-permeable dressing for 24 hours. After removal of dressings and cream in vivo fluorescence was measured at the surface of tumor tissue and adjacent normal skin by means of a spectrofluorometer. Punch biopsies of the same areas were removed and samples were immediately immersed in liquid nitrogen. The tissue sections were cut with a cryostat microtome to a thickness of 8 μm. The localization pattern of the porphyrin fluorescence in the tissue sections was directly observed by means of fluorescence microscopy. The same frozen sections were subsequently stained with routine H&E staining for histological identification. Fluorescence microscopy was carried out with an Axioplan microscope (Zeiss, Germany). Fluorescence images and quantitative measurements were performed by a light-sensitive thermol-electrically cooled charge coupled device (CCD) camera (Astromed CCD 3200, Cambridge, UK) and an image processing unit (Astromed/Visilog, PC 486DX2 66 MHz VL). The main purpose for such quantitative measurements is to determine the exact penetration of ALA-induced porphyrins from tissue surface to the bottom layers of cancer lesions. The results are shown in FIGS. 8 and 9 in which the fluorescence intensity is expressed as a function of depth of cancer lesion.

As shown in FIGS. 8 and 9, an homogeneous distribution of PpIX fluorescence is seen from the top to the bottom of the whole BCC lesions after use of either free ALA or its methyl ester. This suggests that ALA methylester is at least as good as free ALA in terms of penetration and PpIX production in the BCC lesion. In addition, no PpIX fluorescence was seen in the surrounding normal skin after topical application of ALA methylester, indicating that ALA-methylester-induced PpIX highly selectively took place only in the BCC lesion.

In vivo fluorescence after 24 hours showed at least doubled fluorescence intensity for ALA methyl ester compared to ALA for the selected tumors and also an increase for corresponding normal tissues, however this only of about 50%. The ratio between tumor and normal tissue was about 1.2:1 for ALA and 2:1 for the ALA methyl ester. The results are shown in FIGS. 10 and 11.

At control one week after treatment all treatment fields presented a central necrotic area corresponding to the tumor. In the adjacent normal skin exposed to cream and light irradiation there was observed a marked erythema for the ALA while for the ALA methyl ester only moderate erythema was observed.

EXAMPLE 12

In Vivo Fluorescence Surface Measurements of PpIX Production in Human BCC and Surrounding Normal Skin by CCD Microscopy of Biopsies

The present data show the localizatio n patterns and production of porphyrins (mainly protoporphyrin IX (PpIX)) after topical application of free ALA and one of its derivatives (methyl ester) for 4.5 and 24 hours in the nodular basal cell carcinomas (BCCs) and surrounding normal skin of patients. The tests were performed as described in Example 11.

Each of the following figures show both (B) fluorescence images of either the bottom layer of BCC lesions or of the surrounding normal skin. Curves indicating the fluorescence intensity as a function of depth of the BCC lesions or of the normal skin are also shown (A).

FIG. 12 shows a homogenous distribution of PpIX fluorescence generated by ALA methyl ester in the bottom layer of a BCC 4.5 hours after topical application.

There is also some porphyrin fluorescence in surrounding normal skin (FIG. 13). The fluorescence intensity ratio between BCC and the normal skin is about 2. Moreover, the absolute amount of the fluorescence induced by ALA methyl ester is higher than that induced by free ALA and 20% DMSO after topical application for 4.5 hours, as shown below.

FIGS. 14 and 15 show a uniform distribution of porphyrin fluorescence induced by topical application of ALA methyl ester for 24 hours in the bottom layer of BCC and surrounding normal skin. The ratio of the fluorescence in BCC and that in normal skin is also about 2. Furthermore, the fluorescence intensity of ALA methyl ester-induced porphyrins in the BCC is almost twice as high as that in BCC after topical application of free ALA alone for 24 hours, as shown below.

FIGS. 16 and 17 show a homogenous distribution of free ALA-induced porphyrins in the bottom layer of BCC and surrounding normal skin 24 hours following topical application. However, the ratio of the fluorescence intensity between BCC and normal skin is about 1, which indicates a low selectivity of this treatment. Moreover the production of porphyrins in BCC is less than that in the case of ALA methyl ester.

FIGS. 18 and 19 show a homogenous distribution of ALA-induced porphyrins in the bottom layer of BCC and surrounding normal skin after topical application of free ALA and 20% DMSO for 4.5 hours. However, the ratio of the fluorescence intensity between BCC and normal skin is only slightly larger than 1, which demonstrates that the DMSO probably reduces the tumor selectivity of the porphyrins produced. Moreover, also in this case less porphyrins are produced in BCC than in the case of the application of ALA methyl ester.

EXAMPLE 13

Investigation of the Effects of the Chelating Agent Desferrioxamine (DF) and/or DMSO and Fluorescence of Skin

I. The effect of DF and/or DMSO on the build up of fluorescence in the normal skin of mice in situ was ascertained various times after topical administration of ALA-methylester. Methods were performed as described in Example 9.

RESULTS

Topical application of the cream alone containing only DMSO did not show any fluorescence in the normal mouse skin, but there was some fluorescence of PpIX after DF alone was applied.

DF or DF plus DMSO (a well-known skin penetration enhancer) significantly enhanced the production of ALA methylester-induced PPIX.

II. Fluorescence imaging of the skin treated with three derivatives (performed as described in Example 9) showed fluorescence of the ester derivative-induced porphyrins in the epidermis, epithelial hair follicles and sebaceous glands 1 h after topical application (FIG. 21). The fluorescence intensity of the porphyrins increased with the time after the application.

SUMMARY

A large number of patients with basal cell carcinomas (BCCs) has topically been treated with ALA-based PDT in

our hospital during the past five years and more than 90% of superficial BCCs have shown a complete regression. However, nodular BCCs had a low complete response rate due to a poor ALA retention and, consequently, a low ALA-induced porphyrin production in the deep layers of the lesions. In order to improve the technique, we used ALA ester derivatives instead of free ALA. The present data obtained presented in this Example and in Example 9 by means of both fluorescence spectroscopic measurements in situ and fluorescence microscopy of tissue biopsies, indicate that all three ester derivatives studied were taken up, de-esterified and finally converted into porphyrins in the epidermis, epithelial hair follicles and sebaceous glands of the nude mice with a higher porphyrin production than that of free ALA. This is in agreement with the preceding Examples concerning a study of human nodular basal cell carcinoma that demonstrate that the fluorescence of the ALA ester-induced porphyrins was built up faster with a higher intensity and a more homogenous distribution than those of free ALA-induced porphyrins in the lesions.

The present study also shows that DF has a significant effect in enhancing the production of ALA methylester-derived PpIX in the normal skin of the mice after topical application.

Interestingly, a strong fluorescence of free ALA-induced porphyrins was found in regions of the skin outside the area where the cream was topically applied (FIG. 2). This indicates that after topical application free ALA is transported in the blood and porphyrins are subsequently formed in all organs containing the enzymes of the heme synthesis pathway or porphyrins are initially formed in the skin or/and liver, then transported to other tissues via blood circulation. This may lead to skin photosensitivity in areas where free ALA is even not topically applied. However, none of the ester derivatives studied induced porphyrin fluorescence in other parts of the skin.

EXAMPLE 14

Effects of ALA Methylester or ALA, DP and DMSO PDT on Tumor Growth in WiDr Human Colonic Carcinoma-transplanted Nude Mice

Nude mice were transplanted with WiDr human colonic carcinoma cells by subcutaneous injection into the right flank region. The following creams, formulated as described in the preceding Examples, were applied topically to the site of the tumor: 10% DF alone; 20% ALA+10% DF+20% DMSO; or 20% ALA methylester+10% DF+20% DMSO, followed, 14 hours later by laser light irradiation (632 nm, 150 mW/cm^2 for 15 minutes). A separate group of animals bearing the same tumor model, but receiving no topical application of the cream, served as a control. The responses of the treated tumors were evaluated as tumor regression/regrowth time. When the tumors reached a volume 5 times that of the volume on the day of light irradiation, the mice were killed. The results are shown in FIG. 22. (Bars: standard error of mean (SEM) based on 3–5 individual animals in each group). The results show that it took 34 days for tumors treated with ALA methylester+DF+DMSO to reach a volume five times that of the volume on the day just before light irradiation, whereas in the case of free ALA+DF+DMSO it took 24 days for the treated tumors to grow to 5 times size. Thus, ALA methylester is more effective than ALA in slowing tumor regrowth.

EXAMPLE 15

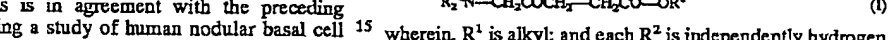
Selectivity of ALA Esters (methyl, hexyl, heptyl and octyl) for Non-normal Tissue

The PpIX fluorescence ratios between BCC lesions and surrounding normal skin after topical application of ALA or its esters (20% for 4 hours), was examined using methods described in previous examples. The results are shown in FIG. 23 and indicate that all esters can more selectively induce PpIX in BCC lesions than free ALA, particularly in the case of ALA-methylester and ALA-hexylester.

We claim:

1. A method for the diagnosis or photochemotherapeutic treatment of disorders or abnormalities of external or internal surfaces of the body, comprising:

i) administering to the sites of investigation or affected surfaces a composition comprising a compound of formula I



(I)

wherein, R^1 is alkyl; and each R^2 is independently hydrogen or alkyl; wherein each alkyl of R^1 and R^2 is optionally substituted by hydroxy, alkoxy, acyloxy, alkoxycarbonyloxy, amino, aryl, oxo or fluoro groups and is optionally interrupted by oxygen atoms; or a salt thereof; and

ii) exposing said sites or surfaces to light.

2. The method of claim 1 wherein aryl is phenyl or a monocyclic 5–7 membered heteroaromatic.

3. The method of claim 1 wherein R^1 represents an unsubstituted alkyl group and each R^2 is hydrogen.

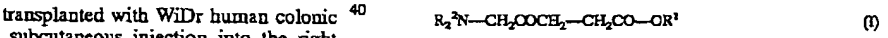
4. The method of claim 1 wherein R^1 represents an unsubstituted alkyl group or each R^2 is hydrogen.

5. The method of claim 1 wherein alkyl contains up to 10 carbon atoms.

6. The method of claim 1 wherein the compound is ALA-methyl ester, ALA-ethyl ester, ALA-propyl ester, ALA-hexyl ester, ALA-heptyl ester, or ALA-octyl ester; or a salt thereof.

7. The method of claim 1 wherein the light is in the wavelength region 500–700 nm.

8. A pharmaceutical composition comprising an effective diagnostic or therapeutic amount of a compound of formula I



(I)

wherein, R^1 is alkyl; and each R^2 is independently hydrogen or alkyl;

wherein each alkyl of R^1 and R^2 is optionally substituted by hydroxy, alkoxy, acyloxy, alkoxycarbonyloxy, amino, aryl, oxo or fluoro groups and is optionally interrupted by oxygen atoms; or a pharmaceutically acceptable salt thereof; together with at least one pharmaceutical carrier or excipient.

9. The composition of claim 8 wherein the compound is ALA-methyl ester, ALA-ethyl ester, ALA-propyl ester, ALA-hexyl ester, ALA-heptyl ester, or ALA-octyl ester; or a pharmaceutically acceptable salt thereof.

10. The composition of claim 8 further comprising at least one surface-penetration assisting agent, and optionally one or more chelating agents.

11. The composition of claim 10 wherein the surface-penetration assisting agent is dimethyl sulfoxide.

12. The composition of claim 10 wherein the compound is ALA-methyl ester, ALA-ethyl ester, ALA-propyl ester, ALA-hexyl ester, ALA-heptyl ester, or ALA-octyl ester; or a pharmaceutically acceptable salt thereof.

13. A method for in vitro diagnosis of abnormalities or disorders by assaying a sample of body fluid or tissue of a patient, said method comprising:

i) admixing said body fluid or tissue with a compound of formula I

$R_2^2N—CH_2COCH_2—CH_2CO—OR^1$ (I)

wherein, R^1 is alkyl; and each R^2 is independently hydrogen or alkyl;

wherein each alkyl of R^1 and R^2 is optionally substituted by hydroxy, alkoxy, acyloxy, alkoxycarbonyloxy, amino, aryl, oxo or fluoro groups and is optionally interrupted by oxygen atoms; or a pharmaceutically acceptable salt thereof;

ii) exposing said mixture to light;

iii) ascertaining the level of fluorescence; and

iv) comparing the level of fluorescence to control levels.

14. The method of claim 13 wherein the compound is ALA-methyl ester, ALA-ethyl ester, ALA-propyl ester, ALA-hexyl ester, ALA-heptyl ester, or ALA-octyl ester; or a pharmaceutically acceptable salt thereof.

15. A kit for use in diagnosis or photochemotherapy of disorders or abnormalities of external or internal surfaces of the body comprising:

i) a first container containing a compound of formula I

$R_2^2N—CH_2COCH_2—CH_2CO—OR^1$ (I)

wherein, R^1 is alkyl; and each R^2 is independently hydrogen or alkyl;

wherein each alkyl of R^1 and R^2 is optionally substituted by hydroxy, alkoxy, acyloxy, alkoxycarbonyloxy, amino, aryl, oxo or fluoro groups and is optionally interrupted by oxygen atoms; or a pharmaceutically acceptable salt thereof;

ii) a second container containing at least one surface penetration assisting agent; and optionally

iii) one or more chelating agents contained either within said first container or in a third container.

16. The kit of claim 15 wherein the compound is ALA-methyl ester, ALA-ethyl ester, ALA-propyl ester, ALA-hexyl ester, ALA-heptyl ester, or ALA-octyl ester; or a phamaceutically acceptable salt thereof.

* * * * *

EXHIBIT C

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
Washington, D.C. 20231
www.uspto.gov

APR 11 2007

Donna M. Praiss
Kenyon & Kenyon
One Broadway
New York, NY 10004

In re: Patent Term Extension
Application for
U.S. Patent No. 6,034,267

NOTICE OF FINAL DETERMINATION -- INELIGIBLE

An application for extension of the patent term of U.S. Patent No. 6,034,267 under 35 U.S.C. § 156 was filed in the United States Patent and Trademark Office on September 22, 2004. The application was filed by PhotoCure ASA, the patent owner of record. Extension is sought based upon the premarket review under § 505(b) of the Federal Food, Drug, and Cosmetic Act (FFDCA) of a human drug product known by the tradename METVIXIA™ having the active ingredient methyl aminolevulinate hydrochloride. METVIXIA™ was approved for commercial use and sale by the Food and Drug Administration (FDA) on July 27, 2004.

A determination has been made that U.S. Patent No. 6,034,267 is **NOT** eligible for patent term extension under 35 U.S.C. § 156 based upon the regulatory review period of METVIXIA™.

A single request for reconsideration of this FINAL DETERMINATION OF INELIGIBILITY may be made if filed by the applicant within TWO MONTHS of the mailing date of this letter. The period for response may be extended pursuant to 37 C.F.R. 1.136. See 37 C.F.R. 1.750. A failure to respond to this letter will result in the application papers being placed into the patent file with no further action taken on the application for patent term extension.

The FDA official records indicate that LEVULAN® was previously approved for commercial marketing or use prior to the approval of METVIXIA™. In a letter dated March 5, 2007, FDA stated:

> A review of the Food and Drug Administration's official records indicates that this product was subject to a regulatory review period before its commercial marketing or use, as required under 35 U.S.C. § 156(a)(4). However, our records also indicate that it does not represent the first permitted commercial marketing or use of the product, as defined under 35 U.S.C. § 156(f)(1), and interpreted by the courts in Glaxo Operations UK Ltd. v. Quigg, 706 F. Supp. 1224 (E.D. Va. 1989), aff'd. 894 F. 2d 392 (Fed. Cir. 1990). The active ingredient in Metvixia, methylaminolevulinate hydrochloride, is an ester of aminolevulinic acid hydrochloride, an active ingredient that has been previously approved for commercial marketing or use as Levulan, NDA 20-965.

Under 35 U.S.C. § 156(a) a term of a patent which claims a product shall be extended if, *inter alia*, the product has been subject to a regulatory review period before its commercial

marketing or use. In addition, under § 156(a)(5)(A):

> the permission for the commercial marketing or use of the product . . . is the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred; (Emphasis added)

Thus, the determination of eligibility of U.S. Patent No. 6,034,267 turns on the provisions in § 156(a)(5)(A) that the permission for the commercial marketing or use is the first permitted commercial marketing or use of the product. The term "product" is defined in 35 U.S.C. § 156(f) as follows:

> (f) For purposes of this section:
> (1) The term "product" means:
> (A) A drug product . . .
> (2) The term "drug product" means the active ingredient of -
> (A) A new drug, antibiotic drug, or human biological product . . . .including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient. (Emphasis added.)

By the explicit terms of section 156(f)(2), the term "product" as it relates to a human drug product means the active ingredient of the new drug product. The active ingredient in the approved product METVIXIA™ is methyl aminolevulinate hydrochloride, which, as an ester of the previously-approved aminolevulinic acid hydrochloride, is by statute is the same product as aminolevulinic acid hydrochloride. As noted in the above FDA letter, the active ingredient LEVULAN® had been approved for commercial marketing and use prior to the approval of the applicant's product. Furthermore, the prior approval of the active ingredient aminolevulinic acid hydrochloride in LEVULAN® by the Food and Drug Administration was under section 505 of the FFDCA, the same provision of law under which regulatory review of the product METVIXIA™ occurred. Applying the definition of "product" provided in section 156(f) to the extension requirement of § 156(a)(5)(A), applicant's product METVIXIA™ does not qualify as the first permitted marketing or use of the active ingredient. Since the approval of METVIXIA™ (methyl aminolevulinate hydrochloride) was not the first permitted marketing or use of the active ingredient thereof, the patent is not eligible for patent term extension based upon the regulatory review of METVIXIA™. See In re Fisons Pharmaceuticals Limited, 231 USPQ 305 (Comm'r Pats. 1986); aff'd, Fisons plc v. Quigg, 8 USPQ2d 1491 (DDC 1988); aff'd, 10 USPQ2d 1869 (Fed. Cir. 1988); Glaxo Operations UK Ltd. v. Quigg, 13 USPQ 1628 (Fed. Cir. 1990).

In view of the above, the term of U.S. Patent No. 6,034,267 is not eligible for extension under 35 U.S.C. § 156 based upon the approval of the product METVIXIA™ and the application for patent term extension, filed September 22, 2004, is dismissed.

Any correspondence with respect to this matter should be addressed as follows:

By mail: Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By FAX: (571) 273-7754

Telephone inquiries related to this determination should be directed to the undersigned at (571) 272-7754.

Kathleen Kahler Fonda
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

cc: Office of Regulatory Policy
HFD - 7
5600 Fishers Lane
Rockwall II Rm. 1101
Rockville, MD 20857

Attention: Beverly Friedman

Re: METVIXIA™ (methyl amino-levulinate hydrochloride)

FDA Docket No. 2007E-0001

IN THE UNITED STATES DISTRICT COURT FOR THE
EASTERN DISTRICT OF VIRGINIA
Alexandria Division

PHOTOCURE ASA,

Plaintiff,

v.

JON W. DUDAS,
Under Secretary of Commerce for
Intellectual Property and Director of
the United States Patent and
Trademark Office,
and JOHN J. DOLL, Commissioner for
Patents,

Defendants.

Civil Action No. 1:08CV718

PLAINTIFF PHOTOCURE ASA'S
DISCLOSURE STATEMENT PURSUANT TO
FED. R. CIV. P. 7.1(a) AND LOCAL CIVIL RULE 7.1(A)(1)

Pursuant to Fed. R. Civ. P. 7.1(a), Plaintiff Photocure ASA ("Photocure") hereby states that (i) it has no parent corporation, and (ii) there are no publicly held corporations owning 10% or more of its stock. Pursuant to Local Civil Rule 7.1(A)(1), Photocure hereby states that (i) a former subsidiary of Photocure (PCI Biotech Holding ASA, which was demerged in June 2008) issued stock to the public in June 2008, and (ii) there is nothing additional to report under Local Civil Rule 7.1(A)(1)(a) and (b).

Respectfully submitted,

Date: July 11, 2008

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